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COMPARATIVE VASOMOTOR REACTIONS IN BRANCHES OF THE ARTERIAL TREE

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From the Laboratory of Physiology of the Northwestern University Medical School

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That arterial pressure varies in different portions of the arterial tree has long been known. Hürthle (1) after a series of experiments on dogs, cats and rabbits came to the conclusion that the mean end pressure in the carotid artery was always slightly higher than that in the femoral artery. Earlier Volkman (2) reported that the systolic end pressure of the femoral artery was greater than that of the carotid artery. Poiseuille (3) early and Dawson (4) later, have shown that the mean pressure is relatively constant throughout the larger branches of the arterial tree. That the systolic end pressure, as measured by the usual methods decreases with the increase in distance from the heart (and with possibly the decrease in the diameter of the artery) is a well known fact and easily demonstrable.

The technique used in this laboratory of taking a series of blood pressure records as an index to the condition of the vasomotor apparatus of an animal, has of necessity demanded the use of a different branch of the arterial tree in each successive determination. Those used have been right and left femoral, right and left subclavian and right and left carotids. The systolic end pressure under the influence of varying doses of adrenin, nicotine and sometimes pituitrin were used to test the vasomotor irritability. Since this systolic end pressure is known to vary in the different arteries, this question arose: does the arterial pressure throughout the arterial tree rise the same number of millimeters of mercury or does the rise bear a constant ratio to the normal systolic pressures occurring in the individual arteries? That the rise is constant

had been assumed as demonstrated but a recent search of the literature has failed to reveal any definite grounds for the supposition. A priori it might be thought that the factors which cause variations in the systolic pressure might cause also a difference in the pressor or depressor responses to drugs. The matter should be determined by direct experimentation. It has been partly answered in this laboratory in a series of experiments by Hoskins and Wheelon (5) from an extended study of the variability of blood pressure and of vasomotor irritability in the anesthetized dog. They, however, took their records from but one artery at a time. They observed that the blood pressure maintained a fairly constant level and that the rises in pressure induced by intravenous injections of pressor drugs were also fairly constant for equal injections. Whether a difference in reaction in the different arteries contributed any variability to their results was not determined

METHOD

To decide the point, normal dogs were anesthetized in the usual manner with ether. Hall reservoir cannulas of essentially the same size were inserted into the different arteries. These were connected by equal lengths of uniform rubber tubing with the large Harvard mercury manometers also of practically the same bore. The mercury columns stood at 12.5 cm. in each manometer and the bases of the manometers were on the same level.

Injections of adrenin, nicotine and pituitrin were used to stimulate the vasomotor system. For the injection of these a venous cannula was connected by a short piece of rubber tubing with a 100 cc. burette containing 0.8 per cent sodium chloride solution. The drug solutions were injected by hypodermic syringe into the rubber tubing above a clamp "shut off" and then run into the vein with 10 cc. of the salt solution.

The blood pressures were recorded on separate drums on which they were measured without any reference to each other. The data were later segregated and compared.

RESULTS

The mean pressures in the different arteries used were found to vary more than the literature on the subject would lead one to expect. They differed usually by from 8 mm. to 40 mm. of mercury. In one case there was a very unusual difference of 120 mm. of mercury in different

arteries of the same dog. This was proved beyond question but the cause was not determined.

In no case was there a significant difference in the amount of rise regardless of whether the rise be sharp or gradual, of a few mm. of mercury or many. The results obtained from one experiment are given below. The pressure noted as "systolic" is the highest indicated by the mercury manometers. The actual damping effect of the instruments was not determined.

Simultaneous rise of blood pressure in different arteries

	RIGHT FEMORAL ARTERY	RIGHT CAROTID ARTERY	SUPERIOR MESENTERIC ARTERY
	Systolic B. P., 96 mm. Mean, 95 mm.	Systolic B. P., 116 mm. Mean, 110 mm.	Systolic B. P., 103 mm. Mean, 102 mm.
	mm.	*mm.*	*mm.*
1 cc. Adrenin	34	34	34
1 cc. Adrenin	40	42	42
2 cc. Adrenin	34	38	38
2 cc. Adrenin	27	26	28
1 cc. Nicotine	48	48	48
1 cc. Nicotine	35	36	35
2 cc. Nicotine	28	30	28
2 cc. Nicotine	29	30	30

Even more striking was the constancy of reaction in the animal which showed the greatest difference in systolic pressure. For instance 2 cc. of adrenin caused the pressure in the carotid artery to rise from 220 mm. to 258 mm. while in the femoral artery it went from 98 mm. to 136 mm. Exactly 38 mm. in each case.

Attempts were then made to determine how far toward the periphery of the arterial branches this constancy persisted. Capillary cannulas were made with which arteries as small as 0.5 mm. in diameter could be utilized.

It was found that if cannulas of the same size were used, one in a branch 0.5 mm. in diameter and another in a branch 5 mm. in diameter, the rises and falls in pressure maintained the same constancy. If, however, the cannulas were not of the same size the rises bore an approximately direct ratio to the sizes of the cannulas.

Later the depressor reactions induced by small doses of adrenin and of the nitrites were compared and found to be as constant as the pressor reactions.

These results show that a sudden rise or fall in arterial pressure is immediately transmitted to all proximal parts of the greater arterial system to the same extent regardless of the existing pressure in the artery from which it is measured.

The outcome of the experiments presents an interesting problem in mechanics. The fact that different systolic and mean pressures exist in various arteries under a constant head of pressure indicates that the peripheral resistance to outflow is variable in the different branches. The augmentation of the resistance produced by the pressor drugs might be expected also to vary in different branches, thus producing a similar difference in the pressor effects, but such is not the case. This fact might be supposed to be correlated with the briefness of the reaction, whereas a longer acting pressor influence might allow an equilibrium to be established and a differing rise sustained. The use of pituitrin, however, shows that such is not the case; that irrespective of the duration of the reaction the pressor effect is the same even when there is a marked difference in the initial pressures.

SUMMARY

Vasomotor reactions, pressor and depressor, in branches of the arterial tree which may be of great variations in diameter and of varying initial systolic and mean pressures, are equal. This can easily be demonstrated provided there is no essential difference in the size of the cannulas used to measure such responses.

I wish to express my sincere appreciation of the suggestions and criticisms of Dr. R. G. Hoskins during the preparation of this paper.

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THE BONE MARROW AS A SOURCE OF PROTHROMBIN

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I. INTRODUCTION

The modern history of blood coagulation begins with the work of Alexander Schmidt in 1861. Since that time a vast literature has grown about the subject, indeed has reached such a size that it is a confusing task to cover the work upon one element in, or one phase of the process. The present investigation concerns itself with the origin of prothrombin from the bone marrow. This substance forms one of the indispensable factors for coagulation. Its necessity is recognized by all theories for the explanation of this process and the technique which we have employed for the demonstration of its presence is equally acceptable to all theories. Morawitz (1, 2), in 1904 and 1905 has reviewed the literature upon the origin of prothrombin or thrombogen or fibrin ferment as the substance is variously called. We shall mention no specific work published prior to these articles, confining ourselves to a statement of the views which existed in 1904 and their modification up to the present date. Such views may be grouped briefly as follows:

(a) Prothrombin arises from all the formed elements of the blood.

(b) Prothrombin arises from the leucocytes.

(c) Prothrombin arises from the platelets:

1. Which are produced by leucocytes and erythrocytes;
2. Which are independent elements.

(d) The formed elements of the blood have nothing to do with clotting.

(e) Prothrombin cannot be obtained from blood free tissue so its source of origin must be in the blood itself.

Formed elements of the blood. Howell (3) in his work on the coagulation of lymph collected from the thoracic duct can not decide finally upon the presence of prothrombin in the lymphocytes. He concludes

that if lymphocytes do contain this substance it is in extremely small amounts. Further evidence against the origin of prothrombin from this source is found in the failure of attempts to obtain it from extracts of lymph glands. Such efforts have been very carefully made by Nolf (4, 5).

Work upon other colorless corpuscles of the blood, mast cells and those indefinite types classed as large mononuclears, has been impossible since these cells can not be collected in sufficient numbers to permit study.

There is no doubt that thoroughly washed mammalian erythrocytes do not yield prothrombin. The nucleated red corpuscles of lower forms are equally deficient. Study of isolated normoblasts of the mammal has been impossible, but in our series of experiments we have found that there is no parallelism between the prothrombin content and the number of normoblasts in the fluids we have tested.

Leucocytes. Morawitz (1) has studied the question of the origin of prothrombin from the leucocytes. After aleuronat injections he examined the washed polymorphonuclear leucocytes so obtained, and found their prothrombin content practically nil. This was the first satisfactory direct examination of these elements, former conclusions being based on the fact that in coagulation there is probably some leucocyte destruction. Nolf (4) also finds no prothrombin in the leucocytes but does get evidence of a large amount of thromboplastic material, which in this particular case he calls leucothrombin, and later thrombozyme (5). Bordet and Delange (6) find a large amount of thromboplastic substance in the leucocytes but nothing that corresponds with any certainty to prothrombin. Their experiments have been confirmed by Lee and Vincent (7).

The question of prothrombin origin from young forms of the leucocytic series has not been adequately investigated. In our own experiments when myelocytes have been numerous in the fluid tested there has been no significant increase in the amount of prothrombin.

Blood platelets. There is a growing conviction that in the adult mammal plates do not arise from leucocytes nor from erythrocytes, but following the work of Wright (8) from the megacaryocytes of the bone marrow. Ogata (9), Bunting (10), Downey (11), and very recently Ferrata and Negreiros-Rinaldi (12), have given Wright's work the most ample histological verification. The absence of prothrombin from erythrocytes and from all forms of white cells encourages this view. Morawitz (1) obtained platelets in pure form by differential centrifugali-

zation and showed them to be rich in prothrombin. Nolf (5) found only a moderate amount of prothrombin in the plates. Bayne-Jones (13) has completely confirmed the work of Morawitz.

Formed elements of blood not concerned in coagulation. These demonstrations—and they might be multiplied—of large amounts of prothrombin in the plates dispose effectively of the idea that the formed elements of the blood have nothing to do with clotting. We know definitely from the work of Deetjen (14) and others that disintegration of the plates accompanies the act of coagulation and indeed is one of the first features in it.

Origin of prothrombin from tissues other than the blood. Nolf (5) and Rettger (15) have found traces of prothrombin in extracts made from the spleen. Rettger obtained what he believed to be a possible trace from washed bone marrow. With the exception of the liver no other tissues have been shown to contain prothrombin. Nolf (16) believes that nearly the whole supply of prothrombin is formed by the liver, basing his belief upon the disappearance of fibrinogen and of prothrombin from the circulation when the liver is removed.

Selling (17) first demonstrated extensive bone marrow destruction in benzol poisoning. Duke (18) found an extremely low platelet count in this condition and ascribed the bleeding tendency to this deficiency. Hurwitz and Drinker (19) carried these observations a step further by finding an agreement between the platelet loss so produced and the quantity of prothrombin in the blood.

We may say in summary, therefore, that prothrombin has been shown to exist in the blood plates, and that these elements originate from the megacaryocytes of the bone marrow. The evidence, however, that the bone marrow itself as a distinct tissue contains prothrombin is very slight. So far as is known other tissues, with the possible exception of the liver and of the spleen, have no significant part in the manufacture of this element.

While devising a method of perfusing bone marrow, the opportunity presented itself of studying the prothrombin content of the perfusing fluid after its passage through the marrow. The discovery of an unexpectedly large amount of prothrombin in this fluid led the authors to make careful studies of specimens of fluid taken at frequent intervals during the course of perfusions lasting 3 to 6 hours, with respect to their content of cells and of prothrombin. The results of these studies, which are reported in detail in this paper, lead to the conclusion that the bone marrow is an important and specialized source of prothrombin.

II. TECHNIQUE

A. Method of perfusion and the collection of specimens

In each experiment the tibia of a dog was perfused through its nutrient artery. A detailed description of the operation used in exposing the nutrient artery and of the method of establishing perfusion is contained in a previous paper (20), so no account will be given here of these features.

The perfusing fluid in some cases was Ringer's solution; in other cases 0.9 per cent NaCl; in still other cases 0.1 to 0.3 per cent $C_2O_4Na_2$ (made up in 0.9 per cent NaCl); and in one case a solution for the preservation of platelets made up according to the formula of Deetjen(14) ($Mn\ SO_4$, 0.5 gm.: NaCl, 0.75 gm.: H_2O_2, 0.5 cc. of 1 per cent solution: H_2O, 100 cc.).

In the majority of the experiments the bone was stripped of muscles, tendons, etc., and removed from the body after the establishment of the perfusion. It was then placed in a closed glass container into the lower end of which a cannula was inserted. Fluid dripping from the bone was collected through this cannula into graduates, which were changed at intervals of 15 to 30 minutes. The specimens so obtained are designated in the protocols as *Specimen 1*, *Specimen 2*, etc., in the order of their collection. Specimens collected in this way became rapidly less bloody, and were soon only faintly colored.

In some of the experiments the bone was not removed from the animal but the specimens were collected by means of a cannula in the popliteal vein. The preparation in these experiments was made as follows. All branches of the popliteal artery and of the lower part of the femoral artery down to and slightly below the origin of the nutrient artery were tied. All structures in the thigh except the femoral artery and vein, the sciatic nerve, and the femur were next ligated and then divided. In order to prevent any loss of blood or of perfusing fluid through the anastomotic circulation around the knee joint and up through the marrow of the femur, the femur was next notched into the marrow cavity and tightly packed with bone wax. By such a dissection all blood leaving the tibia necessarily passed up the popliteal vein and was collected by placing a cannula in this vessel. The arterial cannula was introduced as usual into the popliteal artery below the nutrient artery (20).

B. Method of testing specimens for prothrombin

The method of determining the amount of prothrombin in the various specimens differed according to the perfusing fluid. When Ringer's solution was used the prothrombin tests were made by adding to a given amount of a fibrinogen solution in a series of test tubes, a definite amount of each specimen, and determining the time required for the first signs of clotting; e.g., *fibrinogen*, 0.5 cc.; *specimen 1*, 1.0 cc.; *coagulation time*, 4 minutes. The calcium in the Ringer's solution was sufficient to convert all of the prothrombin in the different specimens into thrombin, which substance then reacted with the fibrinogen to form fibrin. In every experiment each prothrombin test was made in duplicate.

When 0.9 per cent sodium chloride was used as the perfusing fluid it became necessary to add calcium as well as fibrinogen to the specimens before clotting could occur. This was accomplished by adding a certain number of drops of a 0.5 per cent calcium chloride solution to each test tube containing the required amount of fibrinogen, before any of the specimen to be tested was added; e.g., *fibrinogen*, 0.5 cc.; *0.5 per cent* $CaCl_2$, 2 drops; *specimen 1*, 1.0 cc.; *coagulation time*, 9 minutes.

When sodium oxalate made up in physiological saline solution was used as the perfusing fluid, the same procedure of adding calcium chloride to the series of tubes was followed but in these cases it was necessary to add an amount of calcium, sufficient not only to precipitate out all of the oxalate in the solution, but sufficient also to convert all of the prothrombin present into thrombin. The amount of calcium necessary to accomplish these two purposes was determined in each experiment.

The fibrinogen solutions used in these tests were made from the plasma of cats by Howell's modification of the Hammarsten method of precipitation by half saturation with sodium chloride (21). A fresh solution of fibrinogen was made in nearly every case on the day before the experiment, since solutions of isolated fibrinogen deteriorate in a few days. Each solution of fibrinogen was tested immediately before being used with respect to its clotting power when added to a solution of thrombin (22). No fibrinogen solution was used unless 0.5 cc. of it when added to 0.2 cc. of thrombin solution gave a clot in 5 minutes or less.

C. Method of testing for fibrinogen

In one experiment a certain amount of a thrombin solution was added to a definite amount of each specimen of perfusing fluid to determine by the appearance or non-appearance of clots whether or not the specimens contained any fibrinogen; e.g., *specimen*, 0.7 cc.; *thrombin*, 0.2 cc.; *coagulation time*, no clot in four hours. No clots appeared in any case. This led the authors to conclude that the bone marrow is probably not a source of fibrinogen.

D. Other studies made on marrow perfusates

In each perfusion specimen a count was made of the number of red blood cells present and of the number of white cells. Smears and differential counts were also made. Sections were made of the normal and of the perfused bone marrow with the view of studying the effect of perfusion on the histological appearance of this structure. The difficulty, however, of making satisfactory sections of the marrow of the dog because of the cancellous tissue which enmeshes the red marrow, made these histological studies unsatisfactory.

E. Types of control experiments

Three types of control experiments were performed.

1. Experiments to determine the amount of prothrombin demonstrable in normal bloods of various cellular concentrations, with and without the addition of thromboplastic substance.
2. Experiments to determine whether or not solutions dripping over the surface of a fresh naked bone acquire any prothrombin.
3. Experiments to determine whether or not prothrombin is contained in fluids perfused through organs other than the bone marrow.

III. EXPERIMENTAL RESULTS

A. Perfusions with Ringer's solution and with 0.9 per cent sodium chloride

In the early experiments perfusions were made with Ringer's solution, the tibias perfused being stripped of muscles, tendons, etc., and then placed in a glass chamber as previously described. Specimens obtained in this manner all showed the presence of considerable amounts of prothrombin though some bones yielded more than others. The

following table compiled from the results of Experiment XXIII illustrates the usual findings in this type of experiment.

TABLE 1

Experiment XXIII (Ringer's solution)

	SPECIMENS							
	1	2	3	4	5	6	7	8
Prothrombin time in minutes	4	12	28	38	32	46	36	41
Red blood cells per c. mm.	3500	1500	500	600	700	800	850	1650
White blood cells per c. mm.	150	200	90	50	60	100	110	135
Rate of flow in cc. per minute	6.4	6.4	5.9	4.5	3.6	3.0	2.3	1.6

In Ringer perfusates all the prothrombin present is activated to thrombin by the calcium in the solution. In the early specimens which contain a certain amount of blood, this prothrombin may encounter enough fibrinogen to cause the formation of small clots. In this way thrombin is used up and less of it is demonstrable in the collected specimens. For this reason, after using Ringer's solution in a number of cases, it was found more satisfactory to perfuse with 0.9 per cent sodium chloride. Under these circumstances there is no possibility of clotting until the investigator adds both calcium and fibrinogen at the time of making his tests. The sodium chloride perfusates in all cases were rich in prothrombin, as the following table taken from Experiment XI will testify.

TABLE 2

Experiment XI (0.9 per cent NaCl)

	SPECIMENS										
	1	2	3	4	5	6	7	8	9	10	11
Prothrombin time in minutes	9	18	32	47	41	50	53	54	78	44	49
Red blood cells per c. mm.	3400	1700	1500	1320	2300	1400	1200	1000	1600	1300	1800
White blood cells per c. mm.	0	150	200	350	550	260	500	600	500	1200	600
Rate of flow in cc. per minute	8.2	9.8	8.2	3.2	7.2	8.1	8.3	7.6	4.1	6.1	6.0

B. Control experiments: possible sources of prothrombin other than the marrow

(1) *Blood.* In an examination of these tables the question naturally arises—Is the small amount of blood which these specimens contain the source of the prothrombin in them? In Experiment XI (table 2) blood was allowed to flow from the carotid artery of the dog whose tibia was being perfused into a large volume of 0.9 per cent sodium chloride until the color of the salt solution closely resembled that of the first specimen of perfusing fluid.

A count was then made of the number of red cells in this diluted blood and a determination of the time required for clotting after the addition of a given amount of calcium chloride and of fibrinogen—this latter maneuver constituting our usual test for prothrombin; e.g., *fibrinogen*, 0.5 cc.; *0.5 per cent* $CaCl_2$, 2 drops; *diluted blood*, 1.0 cc.; *coagulation time*, no clot in 3 hours, 49 minutes.

The number of red cells per c. mm. in the diluted blood was 3500: the prothrombin test gave no clot in 3 hours and 49 minutes. The first specimen of marrow perfusate taken from the same dog showed 3400 red cells per c. mm., approximately the same number as in the diluted blood; while the prothrombin test in this specimen gave a clot in 9 minutes.

We next prepared from Dog XI a second lot of diluted blood in which the red cell concentration was determined to be 25,320 cells per c. mm. The prothrombin time in this case was 34 minutes. These figures in their turn should be contrasted with the red cell count of 3400 per c. mm. and the prothrombin time of 9 minutes in the first specimen of the perfusing fluid.

These observations on diluted blood furnish definite evidence that the small amounts of blood present in the specimens of fluid which have passed through the marrow are not the source of the prothrombin in them.

In order to furnish still further evidence on this point Experiment XIII was performed in which a given amount of oxalated blood was diluted with 0.9 per cent sodium chloride through a series of progressively increasing dilutions. At each dilution a red cell count and a white cell count was made, and the prothrombin time was determined in the usual manner. In this way we determined approximately the normal prothrombin times for bloods of different cellular concentrations. When these normal prothrombin times are compared with the prothrom-

bin times of specimens of Ringer's solution or of 0.9 per cent sodium chloride which have passed through the marrow, it will be found in every case that the marrow perfusates contain much greater amounts of prothrombin.

TABLE 3

Experiment XIII (Blood diluted with 0.9 per cent NaCl)

	SPECIMENS									
	1	2	3	4	5	6	7	8	9	10
Dilution: Blood to 0.9 per cent NaCl	1: 10	1: 20	1: 40	1: 80	1: 160	1: 320	1: 640	1: 1280	1: 2560	1: 5120
Prothrombin time in minutes	3	5	8	16	37	69	153	no clot in 6 hrs.	no clot in 6 hrs.	no clot in 6 hrs.
Red blood cells per c. mm	462,000	261,000	94,640	66,000	31,800	14,648	10,348	5748	4448	3450
White blood cells per c. mm	1496	848	340	200	70	15	4	0	0	0

Table 3 summarizes the figures obtained in Experiment XIII. But it must be remembered that in many of the experiments the specimens of perfusing fluid dripped over the surface of the bone before they were collected. In this way each specimen received an increment of thromboplastic substance.

In order to simulate this state of affairs in diluted blood a second lot of oxalated blood was diluted with water in a series of dilutions corresponding to the 0.9 per cent sodium chloride dilutions just described. The water at once caused complete hemolysis with the liberation of large amounts of thromboplastic substance. Since it was not possible in this series to count the number of red cells at each dilution, the concentration of blood in each specimen was estimated from the corresponding sodium chloride specimen.

The prothrombin times in the water diluted bloods were then tested in order to determine the effect of the increment of thromboplastic material on the rate of clotting. The figures obtained in this series may be seen in table 4.

But even these shorter prothrombin times contrast markedly with the still shorter prothrombin times in marrow perfusates of the same red cell concentration. Take for example in table 4 the specimen of

TABLE 4

Experiment XIII (Blood diluted with H_2O)

	SPECIMENS									
	1	2	3	4	5	6	7	8	9	10
Dilution: Blood to H_2O	1: 10	1: 20	1: 40	1: 80	1: 160	1: 320	1: 640	1: 1280	1: 2560	1: 5120
Prothrombin time in minutes	1.5	2	3	4	5	12	21	41	77	222
Estimated red blood cells per c. mm.	462,000	261,000	94,640	66,000	31,800	14,648	10,348	5748	4448	3450
Estimated white blood cells per c. mm.	1496	848	340	200	70	15	4	0	0	0

water diluted blood which contained 3450 red cells per c.mm. The prothrombin test on this blood gave a clot in 222 minutes. Perfusion specimen 1 of Experiment XI contained 3400 cells per c.mm.; the prothrombin time was 9 minutes. These observations seem to us to prove conclusively that while the dilute blood present in the perfusion specimens undoubtedly adds a trace of prothrombin to these liquids, it is certainly not the chief source of the prothrombin which they contain.

(2) *Surface of the bone.* After eliminating the blood as the chief source of the prothrombin in the marrow perfusates, we next investigated the question as to whether or not salt solution which has not passed through the marrow but which has dripped over the surface of a fresh bone, contains any prothrombin. Experiments on this point demonstrated that a solution dripping slowly over a freshly stripped bone picks up small amounts of blood and in this way acquires traces of prothrombin. That the amounts of prothrombin acquired in this way are very small may be seen from table 5 (Exp. XV) which should be contrasted with table 2 (Exp. XI), showing the prothrombin present in marrow perfusates.

In order to eliminate entirely the factors involved when the perfusing fluid drips over the bone, a series of experiments was performed in which the bone was not removed from the animal after the establishment of perfusion. The technique of this preparation has already been described. The disadvantage to this method of collection is the fact that the specimens are apt to contain more blood than specimens obtained in the manner first described. This disadvantage prevented us from using exclusively this method of collection. The

TABLE 5

Experiment XV (0.9 per cent NaCl allowed to drip over bone)

	SPECIMENS					
	1	2	3	4	5	6
Prothrombin time in minutes	40	1 hr. 42 min.	no clot after 2 hrs. 47 min.	no clot after 2 hrs. 27 min.	no clot after 2 hrs. 17 min.	no clot after 2 hrs. 3 min.
Red blood cells per c. mm.	4400	848	448	50		
White blood cells per c. mm.	50	14				
Rate of flow in cc. per minute	4.3	7.0	8.6	3.6		

results obtained from prothrombin tests on perfusing fluids collected directly from the popliteal vein showed that these liquids contained a quantity of prothrombin greater than could possibly be accounted for by the amount of blood in them. The following table taken from Experiment XVI gives a series of figures illustrating this fact.

TABLE 6

Experiment XVI (0.9 per cent NaCl—Specimens collected from vein)

	SPECIMENS														
	1	2	3	4	5	6	7	8	9	10	11*	12	13	14	15
Prothrombin time in minutes	14	30	13	7	5	9	11	4	16	20	11	37	28	21	28
Red blood cells per c. mm.	61,800	38,800	16,800	22,960	19,440	10,960	9280	7000	5648	2248	20,160	5240	4240	4000	3600
White blood cells per c. mm.	150	250	700	500	600	400	300	250	200	250	700	550	200	548	250
Rate of flow in cc. per minute	7.2	6.0	3.0	1.4	1.2	1.3	1.3	1.3	1.2	1.3	1.7	1.0	1.2	1.3	1.5

*This specimen contained a clot which had caught in the rubber tubing and had collected cells around it.

(3) *Other organs. Prothrombin content of 0.9 per cent sodium chloride perfused through liver and spleen.* In order to determine whether or not liquids perfused through organs other than the bone marrow show a content of prothrombin, perfusions were made of the spleen of a dog and of the liver of a guinea pig and the perfusing fluids examined for prothrombin. The results in the case of the spleen were negative.

The traces of prothrombin found could be accounted for by the blood present. In the liver on the contrary a certain small amount of prothrombin was found which could not be due to blood.

C. Possible sources of prothrombin obtained from bone marrow

The question which next arose in this series of experiments was—What cells in the bone marrow are the source of the prothrombin? In attempting to answer this question we considered as a possible source each of the following groups of cells:

(*1*) *Leucocytes.*
(*2*) *Lymphocytes.*
(*3*) *Myelocytes.*
(*4*) *Nucleated red cells.*
(*5*) *Megacaryocytes.*
(*6*) *Blood platelets.*
(*7*) *Wandering endothelial cells.*

(1, 2, 3, 4) *Leucocytes, lymphocytes, myelocytes, nucleated reds.* Of these seven groups of cells we easily eliminated the first four as possible sources of prothrombin. In nearly all of our experiments portions of the perfusion specimens were centrifugalized and smears made from the sediments obtained. Differential counts on these smears gave the percentage of the different types of cells present in each specimen. By this means we were able to compare the amounts of prothrombin present with the number of cells of each variety found. We were never able, however, to establish the least parallelism between the amounts of prothrombin present and the number either of leucocytes, lymphocytes, myelocytes, or nucleated red cells. A specimen rich in leucocytes, for example, was often rich in prothrombin, and equally often its prothrombin content was exceedingly low. That these results agree with the work of other investigators may be seen by referring to our introductory discussion of the theories of origin of prothrombin.

(5, 6) *Megacaryocytes and blood platelets.* The possibility of the origin of the bone marrow prothrombin from the megacaryocytes or the platelets deserves careful consideration. According to the work of Wright (8) and others, the platelets arise as buds from the megacaryocytes, and it has been shown that solutions of platelets contain large amounts of prothrombin. We are, therefore, at once confronted

with the question—Does the prothrombin which we have found come from the mother megacaryocytes, from the daughter platelets, or from both?

Our first inclination was to suspect the platelets, which we believed had probably disintegrated during perfusion and had yielded their prothrombin to the perfusion liquids. In endeavoring to find proof for this idea we perfused bones with sodium oxalate solutions (made up in 0.9 per cent NaCl), varying in oxalate strength from 0.1 per cent to 0.3 per cent, in the hope of fixing the platelets and so preventing their disintegration. If such a manoeuver were successful, one ought to find no prothrombin in the oxalate perfusion liquids, or at least only traces, and at the same time plenty of fixed plates. As a matter of fact in all the oxalate perfusates (except the 0.1 per cent perfusate which seemed to be too weak to fix the cells), we did find practically no prothrombin. But at the same time the most careful search revealed no fixed plates, although the usual number of red and of white cells appeared in the perfusion specimens. Centrifugalization of these specimens and treatment of their sediment with water (a manoeuver which would cause fixed platelets to disintegrate and yield prothrombin) gave negative results to our prothrombin tests.

These findings seem to point to one of two conclusions: either (a) the platelets are not the source of the marrow prothrombin, or (b) if the platelets are the source of the marrow prothrombin they are fixed in situ by oxalate solutions and are not washed out with the other marrow cells during perfusion.

Further experience with oxalate solutions showed that if these perfusions are continued long enough prothrombin will eventually appear in the specimens. This suggests that the cells of origin of prothrombin are at first fixed in situ by the oxalate but that under the influence of prolonged washing they will eventually go to pieces. Experiments were performed in which bones were perfused with 0.9 per cent sodium chloride for an hour or more and then the perfusing fluid was suddenly changed to an oxalate solution. In an experiment of this sort the saline perfusate contained the usual prothrombin. This prothrombin promptly disappeared when perfusion with an oxalate solution was begun. The reverse type of experiment was performed in which the bone was first perfused with an oxalate solution and then with physiological saline. The results were the same. The oxalate specimens contained no prothrombin while the saline specimens contained gradually increasing amounts as the salt solution slowly washed

the oxalate out of the cells in the marrow and brought about their disintegration. The figures obtained in an experiment of this type may be seen in table 7 taken from Experiment XXVI.

TABLE 7

Experiment XXVI (0.2 per cent $C_2O_4Na_2$ changed to 0.9 per cent NaCl)

	SPECIMENS									
	1	2	3	4	5*	6	7	8	9	10†
Prothrombin time in minutes	no clot in 4 hrs.	no clot in 4 hrs.	no clot in 4 hrs.	no clot in 4 hrs.	1 hr. 40 min.	1 hr. 21 min.	1 hr. 9 min.	55 min.	33 min.	18 min.
Red blood cells per c. mm.	6900	5600	4800	1300	1350	900	1200	1000	1100	3300
White blood cells per c. mm.	4000	2500	1300	500	100	250	50	50	43	3100
Rate of flow in cc. per minute.	8.4	6.0	3.8	2.2	2.1	2.1	2.1	2.1	1.8	3.3

* 0.9 per cent sodium chloride perfusion begun.

† The appearance of fat droplets in this specimen indicated a general disintegration of the marrow.

Smears made from the sediment of specimens in this particular experiment showed but one megacaryocyte in the first oxalate specimen but several intact megacaryocytes and large numbers of naked nuclei of megacaryocytes in the saline specimens. This suggested that possibly the prothrombin found in our perfusion specimens came from the disintegration of megacaryocytes and not from the plates. The results of the oxalate perfusions fit in well with such an idea. It seems highly improbable that small elements like the plates if present in large numbers should not be washed out of the marrow by the oxalate solutions when myelocytes, leucocytes, and erythrocytes come out readily under such circumstances. The megacaryocytes on the other hand are large branching cells with processes extending into the marrow reticulum. It is reasonable to suppose that such cells would be fixed in place by an oxalate solution and could be broken up only by prolonged washing or by changing the perfusing fluid to some solution which did not act as a fixative. On the other hand initial perfusion with Ringer's solution or with 0.9 per cent sodium chloride might readily cause disintegration of cellular processes extending from the megacaryocytes into the vascular channels, and a consequent liberation of prothrombin into the perfusing liquid. This idea is advanced merely as a suggestive possibility.

(7) *Endothelial cells.* The possibility that the marrow prothrombin might arise from wandering endothelial cells was also considered. In investigating this possibility we stained one animal intravitally with trypan blue. One tibia of this animal was perfused with oxalate and the other with saline solution. The usual results were found as far as prothrombin was concerned. The oxalate specimens contained practically no prothrombin while the saline specimens were rich in this substance. Smears made from the sediments of the different specimens showed no intravitally stained cells in either the oxalate or the saline perfusates. Smears made after perfusion from the marrow tissue of both tibias, however, showed plenty of stained endothelial cells. From these observations one must conclude either (a) that wandering endothelial cells are not the source of the marrow prothrombin, or that (b) if they are a source of prothrombin they are neither disintegrated nor washed out of the marrow during perfusion but yield their prothrombin by a process of secretion. The former conclusion seems the more probable.

In summing up briefly the situation in regard to the cells of origin of the marrow prothrombin, we may say that there is no evidence that the prothrombin in question comes either from leucocytes, lymphocytes, myelocytes, nucleated red cells, or wandering endothelial cells. On the other hand the blood platelets are known to contain prothrombin, and the origin of the plates from the megacaryocytes of the marrow is well established. In the course of the work reported in this paper the authors have performed experiments in which bones perfused with 0.9 per cent sodium chloride or with Ringer's solution yielded large amounts of prothrombin while the sediment from such perfusion liquids contained practically no megacaryocytes. On the other hand bones perfused with sodium oxalate solutions yielded practically no prothrombin, but the perfusion liquids in these cases showed intact megacaryocytes and large numbers of naked nuclei of megacaryocytes. These facts all point to the megacaryocytes as a probable source of the marrow prothrombin.

CONCLUSIONS

(1) Evidence is presented that fibrinogen is not formed in the bone marrow.

(2) Prothrombin is formed in large amounts in the bone marrow.

(3) This prothrombin does not arise either from leucocytes, lymphocytes, myelocytes, nucleated red cells, or from wandering endothelial cells.

(4) The megacaryocytes are the probable source of the marrow prothrombin.

This work was undertaken at the suggestion of Dr. W. H. Howell, and we are greatly indebted to him for his constant interest and advice.

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EXPERIMENTS WITH OIL OF CHENOPODIUM AND CARDIAC STIMULANTS ON THE ISOLATED FROG HEART

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In a previous communication from this laboratory (1) it was shown that oil of chenopodium or ascaridol when injected intravenously into different animals caused depression of the circulation.

In continuation of these studies with the oil and its active principle, experiments were carried out on the isolated frog heart, special reference being had to antagonistic action for which purpose Adrenalin, Digitalis and Caffeine were employed. These drugs were perfused either in combination with oil of chenopodium or after the heart had been exposed to its action long enough to produce a pronounced effect.

The method is shown in figure 1. The oil of chenopodium or ascaridol, was shaken up with Ringer's solution and passed through filter paper, but later in the course of the investigation it was found advisable to add neutral olive oil to the perfusing fluid. This mixture was agitated for some time so as to make a fine suspension and was filtered before using.

When the fresh heart, soon after its excision, was perfused with oil of chenopodium, or ascaridol, in Ringer's solution no change in its action could be observed. In nearly every case the result of the first test proved negative, although the perfusion time as well as the amount of chenopodium added to the solution varied in many instances. The initial trial with 1 to 2 minims of ascaridol or oil of chenopodium in 100 cc. of Ringer's solution perfused for one-half to one minute failed to produce any change in cardiac activity. In some experiments, no signs of decreased action of the heart could be observed for a considerable time after its removal from the body, a number of perfusions with oil of chenopodium be-

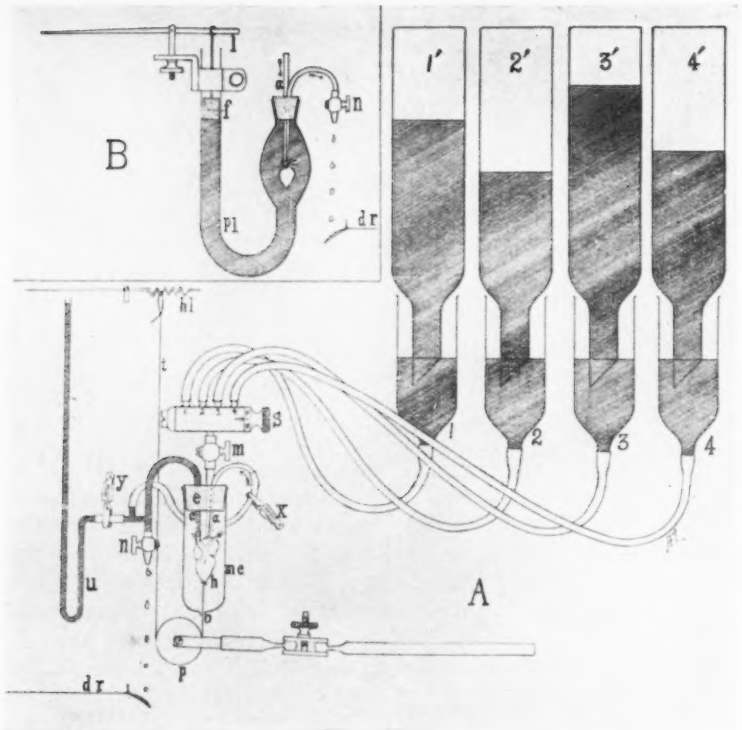


Fig. 1. A diagram of a modified form of frog heart perfusion apparatus described by Simpson (2) is shown in *A*. The double brass cannula (*a*) was inserted into the sinus and the glass cannula (*c*) into the aorta (heart *h*). Both cannulae are firmly fixed in a piece of cork (*e*) over which the moist chamber (*mc*) fits. Before the moist chamber is put in place a thread (*t*), fastened to the apex of the ventricle is passed through the opening (*o*) and around a pulley (*p*) to the short arm of a Harvard heart lever (*hl*). Perfusion from any one of the four reservoirs (*1, 2, 3, 4*) may be made by turning the milled head of the Macmillan (3) stopcock (*s*) to the corresponding number. The four reservoirs (*1, 2, 3, 4*) which automatically keep the fluids at the same level, together with the supply flasks (*1′, 2′, 3′, 4′*) are held in a rack which may be vertically adjusted so as to provide any desired inflow *pressure*. The *rate* of inflow may be regulated by stopcock (*m*) and the outflow by stopcock (*n*). A Macmillan (4) drop recorder (*dr*) is used to register the outflow in drops. When it is desired to circulate the same small quantity of fluid continuously for some time stopcocks (*m*) and (*n*) are closed and clamps (*x*) and (*y*) are removed. The U-tube (*U*) allowed to fill to any height with the same fluid, shows the pressure against which the heart is working.

In some experiments the volume method was used as shown diagrammatically in *B*. In this case the double brass cannula (*a*), connected to the perfusion apparatus as in *A*, was inserted directly into the ventricle, one side serving as inflow and the other as outflow. The ventricle is immersed in the Ringer's fluid which completely fills the plethysmograph (*pl*). The variation in level of the fluid is transmitted by the float (*f*), to the recording lever (*l*).

ing carried out without causing any disturbance of function. As a rule, the fourth to the sixth perfusion caused depression, the period of exposure in each case being not longer than a half minute. Exceptionally, the effect may become manifest with the second perfusion. In one case, on the contrary, a very slight depression only was first noticed as a result of the twelfth trial. It may be pointed out in this connection that when the heart became susceptible to

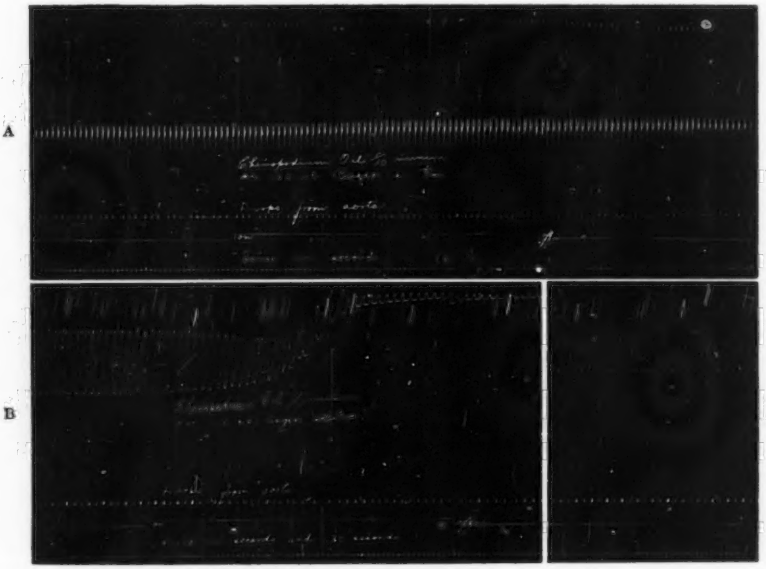
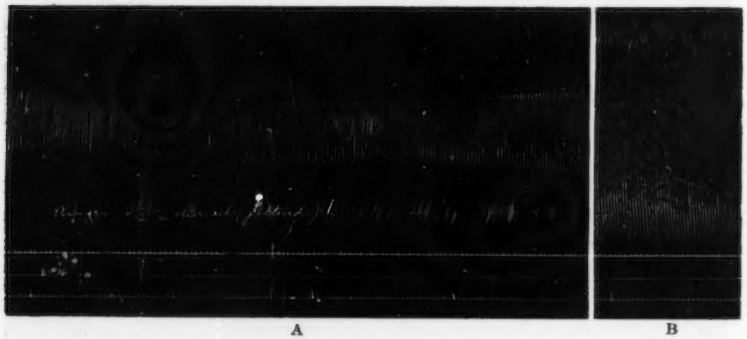


Fig. 2. *A*, Shows action of oil of chenopodium on the fresh heart. *B*, One hour after it had been perfused several times with oil of chenopodium. Heart in *diastole* (overdistended with fluid causing shortening of vertical diameter). *C*, Shows heart action twelve minutes after *B*.

oil of chenopodium its effect generally increased with each successive perfusion until cardiac activity was temporarily (see fig. 2), and sometimes permanently abolished. The result seemed to be independent of the amount suspended in the perfusion fluid. One minim, and in some instances even a much smaller quantity, added to 100 cc. of Ringer's solution produced this effect (see fig. 2). It might further be stated that the action seemed to be independent

also of the frequency and rate of perfusion, as the same was noticed after intervals varying between 3 and 12 minutes. This may be explained by the fact that oil of chenopodium is very sparingly miscible in water. It is quite possible that the saturation point was reached when very small amounts of oil of chenopodium were employed.

In order to make comparable tests with different amounts, a suspension of olive oil in Ringer's solution was resorted to as a suitable medium for dissolving the oil of chenopodium, thus enabling us to study the effect of concentration. One half cc. of neutral olive oil was added to 100 cc. of Ringer's solution and thoroughly shaken, then filtered and the desired amount of oil of chenopodium was



A B

Fig. 3. Frog 109. *A*, General improvement of heart action when perfused with Ringer olive oil following perfusion with Ringer's solution. *B*, Effect of Ringer olive oil twelve minutes after *A*.

added and again agitated. Control tests made to ascertain the action of olive oil showed at once that this was a very powerful stimulant whose action was particularly striking when the heart became weak as a result of prolonged perfusion with Ringer's solution. In some experiments the amplitude gradually increased until it reached three times its initial strength, moderate augmentation of rate being observed at the same time (fig. 3). The effect could be easily demonstrated by perfusing the heart alternately with Ringer's solution alone and with Ringer olive oil. Stimulation set in soon after the Ringer olive oil reached the heart and ceased when perfusion with Ringer's solution was resumed. The action of olive oil was observed about half a minute after perfusion was

begun and remained uniform after reaching a maximum as long as perfusion with it was continued. It may be recalled that similar effects were obtained by Clark (5) with sodium oleate and other soaps of the higher fatty acids in the hypodynamic heart which is a con-

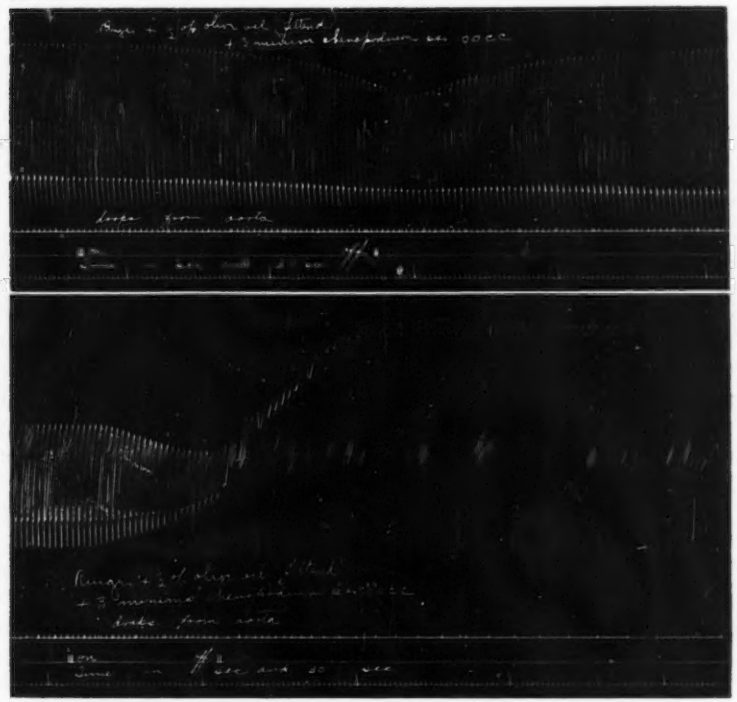


Fig. 4. Frog 119. *A*, First perfusion shows moderate effect of oil of chenopodium on the fresh heart. Perfusion time, one minute. *B*, Seventh perfusion. The same amount oil of chenopodium seventy-five minutes later, perfusion time about half. Heart in diastole (overdistended with fluid causing shortening of vertical diameter and apparent systole).

dition of diminished activity established after it has been perfused for some time with Ringer's solution. When 3 minims of oil of chenopodium in 100 cc. Ringer's solution containing olive oil were perfused for 1 minute moderate depression, from which it soon recovered, was observed. Depression was sometimes produced,

however, with one minim of oil of chenopodium to 100 cc. of R+ olive oil. The action was more marked when the length of the perfusion time was increased. Thus, in two experiments in which 3 minims oil of chenopodium per 100 cc. were perfused 2 to 2½ minutes, heart action became very feeble in one case and almost disappeared in the other 1½ minutes after it was discontinued. In three other experiments in which perfusion lasted 3½, 4½ and 5 minutes, arrest of cardiac action was observed 3 minutes after the test with oil of chenopodium was begun. The effect was different with smaller amounts. Thus, whereas slight depression was produced with one minim to 100 cc. fluid when perfused for one minute, a decrease in rate varying between 60 and 70 per cent and a slight diminution of amplitude was noticed when the perfusion time was increased to 6 minutes.

The results with different amounts were very striking with the plethysmographic method of perfusion. One minim of oil of chenopodium to 100 cc. Ringer's solution and olive oil caused arrest of the heart in diastole for about 20 seconds; 1½ minims suspended in the same amount of fluid stopped the heart for more than 2 minutes, the length of perfusion time in each case being one minute. Although the dose and perfusion time are important elements in determining the action of oil of chenopodium on the isolated heart other factors, as already pointed out, must be taken into consideration.

The number of perfusions seemed to play an important rôle also in these experiments as the second or third perfusion with oil of chenopodium sometimes produced a much greater effect than the first. Temporary and sometimes permanent cessation of cardiac activity was observed after the seventh, eighth, or even after the sixth perfusion (figs. 4 and 5), 30 to 75 minutes after tests on the heart were begun. It is worthy of notice that the intervals between the tests varied between 5 and 47 minutes. That would indicate that the action of oil of chenopodium is cumulative. Evidence obtained in a number of experiments suggests, however, that the condition of the heart at the time oil of chenopodium is applied may be a strong causative factor in determining the action.

Thus the maximum effect was very often independent of the number and even of the frequency of perfusions with oil of chenopodium. In one experiment with 1½ minims to 100 cc. fluid the heart stopped after the third perfusion which lasted 1½ minutes, the intervals being 7 and 4 minutes. In another experiment with the same amount the heart stopped after it was perfused for 30 seconds, only one test

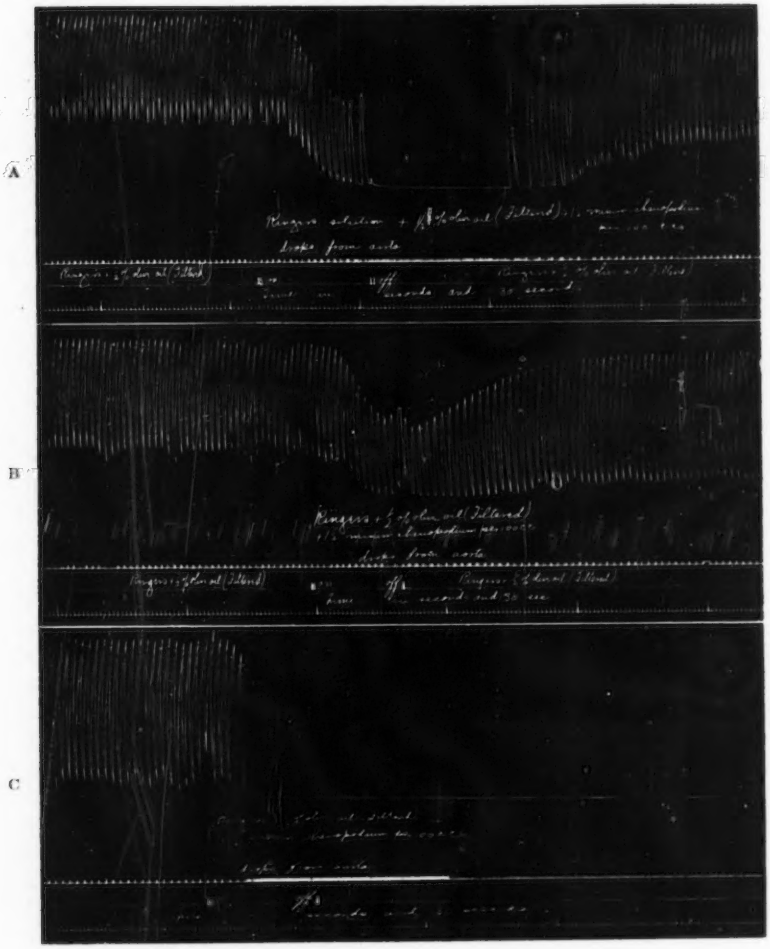


Fig. 5. Frog 85. Plethysmographic method. Ventricle only perfused. *A*, Effect of oil of chenopodium on fresh heart. *B*, Third perfusion, six minutes after *A*. *C*, Sixth perfusion, twenty-three minutes after *B*. Heart died.

with oil of chenopodium being made 25 minutes previously, the perfusion time in this instance being also 1½ minutes. In a third experiment the heart stopped with the first trial, the perfusion time being again 30 seconds. Only one minim oil of chenopodium was used. It may be observed that previous to perfusion with oil of chenopodium the heart had been perfused with olive oil in Ringer's solution for 15 minutes. A similar observation was made by Clark (6) in experiments with strophanthin. The reaction of the heart of the frog held in captivity showed a condition similar to that of the hypodynamic heart. That increased sensitiveness to oil of chenopodium may be compatible, however, with vigorous heart action was very strikingly illustrated in several experiments. In one case the contractions were uniform and vigorous immediately before the oil of chenopodium treatment, yet it proved to be very toxic, as the heart stopped almost immediately after the first trial with the drug which was tested after 15 minutes perfusion with olive oil. These variations in the resistance to oil of chenopodium were observed when the heart was perfused through the venous sinus as well as in experiments in which the plethysmographic method was used.

EXPERIMENTS WITH ADRENALIN

When the frog heart was perfused with Ringer's solution and olive oil containing oil of chenopodium and adrenalin, cardiac action in most experiments either remained normal or was distinctly stimulated, a marked increase in force of about 60 per cent and moderate augmentation of frequency being observed as a result of the treatment. The effect of adrenalin sometimes persisted several minutes after the perfusion. Occasionally, however, antagonistic action was absent, which may be accounted for by the rapid oxidation of adrenalin in oil of chenopodium, frequently observed in the course of our experiments. That it was not due to loss of potency of the preparation was shown by the reaction of the heart to adrenalin in Ringer's solution and olive oil without oil of chenopodium, as well as by blood pressure tests on rabbits. It might also be added that all the tests with oil of chenopodium which failed to respond to adrenalin were carried out in the early stages of the experiment. It is worthy of notice that the fresh heart was less sensitive to adrenalin as well as to oil of chenopodium than a heart which has been perfused for some time with Ringer's solution.

A series of experiments was also conducted, in which adrenalin was tried after oil of chenopodium in Ringer olive oil emulsion was perfused until heart action stopped or exhibited signs of considerable injury. The results established conclusively the antagonistic action of these two substances as shown in the following experiments. Condensed protocols are presented below.

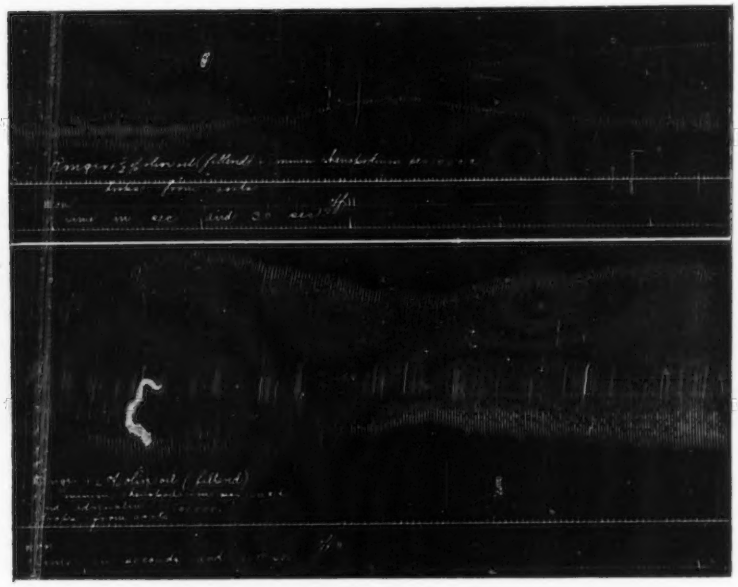


Fig. 6. Frog 103. *A*, Action of oil of chenopodium. *B*, Shows antagonistic action of oil of chenopodium and adrenalin.

Frog 102. One minim oil of chenopodium suspended in 100 cc. Ringer's solution and olive oil perfused for a half minute produced a moderate decrease of amplitude with some diminution of frequency from which it promptly recovered after the application was discontinued. Practically the same effect was observed 12 minutes later when such a solution containing 1: 100,000 adrenalin was perfused for an equal period of time. Adrenalin alone, however, produced the usual effect on the heart as was shown by a previous perfusion. Another perfusion with the same amount of oil of chenopodium for 25 seconds made four minutes later rapidly produced marked cardiac depression followed by pronounced irregularity of action. No improvement having occurred in 3½ minutes, perfusion with 1: 100,000 adrenalin in Ringer's solution and olive oil for one minute was

resorted to. Within 20 seconds heart action became vigorous and regular, continuing in this condition for about 10 minutes.

Frog 169. The heart has been arrested in diastole, when perfused for one minute with 3 minims oil of chenopodium. A return of activity occurred almost immediately after the test with adrenalin was begun, the heart having been in a condition of paralysis 1¼ minutes. The contractions were at first feeble but they gradually increased.

Frog 170. Perfusion with 3 minims oil of chenopodium to 100 cc. Ringer's solution. Depression set in within two minutes, continued 3 minutes until the heart stopped. Perfusion with 1: 100,000 adrenalin made two minutes later had no effect. The experiment was repeated with a very strong solution of adrenalin 26 minutes after oil of chenopodium. Well marked and regular contractions appeared almost immediately and lasted 10 minutes. The heart continued to beat 15 minutes longer and died. The reviving action of adrenalin was also observed in another experiment.

Fig. 7. Frog 169. Shows reviving action of adrenalin on heart arrested in diastole by oil of chenopodium.

Frog 172. In this case the heart was perfused with the same concentration of oil of chenopodium for 3¾ minutes. Cardiac action ceased in 2¾ minutes. A solution of 1: 100,000 adrenalin was perfused immediately after oil of chenopodium. Feeble contractions appeared within half a minute. Such prompt action was, however, not always obtained. That a longer interval may elapse between the application of adrenalin and return of cardiac activity is shown in the following experiment.

Frog 171. Heart perfused with 3 minims oil of chenopodium to 100 cc. fluid was arrested in diastole after 2½ minutes exposure to its action. Perfused 4½ minutes. One and one-half minutes later perfusion with 1: 25,000 adrenalin was begun and was continued 14 minutes Feeble contractions of the heart appeared within 3 minutes and steadily increased in force for 11 minutes until heart action was almost normal and remained in this condition. Several minutes after adrenalin was discontinued, heart action ceased but contractions reappeared when it was perfused again with adrenalin.

EXPERIMENTS WITH DIGITALIS

The official tinctures were used in these experiments, concentrations of 0.1 to 1 per cent being employed. The antagonistic action to oil of chenopodium was also observed with digitalis but the com-

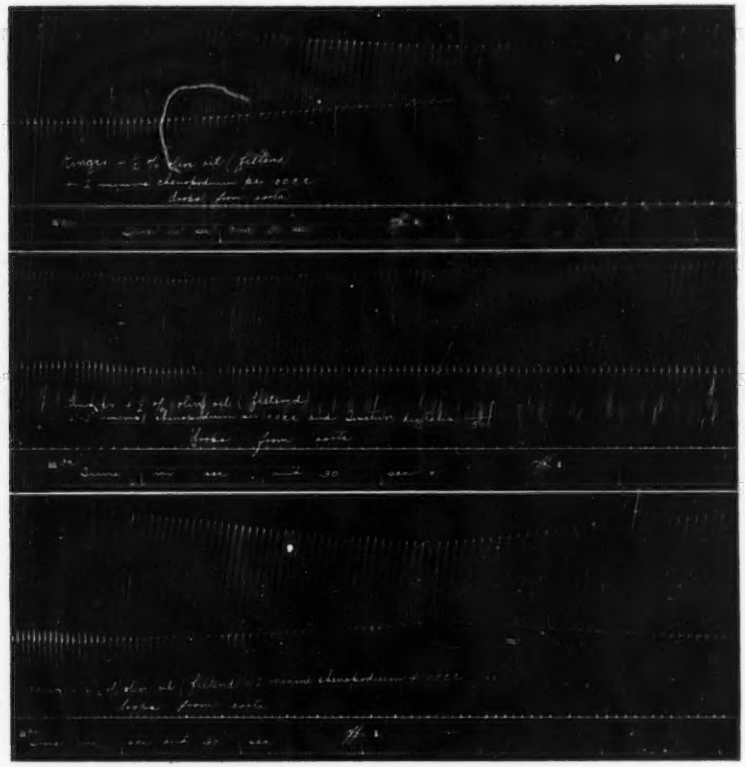


Fig. 8. *A*, The effect of oil of chenopodium. *B*, Antagonistic effect of digitalis and oil of chenopodium. *C*, Seventeen minutes after *B*. Previous treatment with digitalis decreases the action of oil of chenopodium.

bined effect of the two drugs varied. Stimulation was observed in some cases, the effect in others being just sufficient to neutralize the action of oil of chenopodium, while in some experiments cardiac

depression, though slight, could be observed; in such cases a stimulating after effect lasting 5 to 7½ minutes occurred. It is worthy of notice that the effect of oil of chenopodium was less marked when preceded by treatment with digitalis. Thus in one experiment decreased action of oil of chenopodium was observed 17 minutes after it was perfused with digitalis, which may be explained by recent studies on the cumulative action of the digitalis bodies. Hatcher (7) has shown that the action of members of this group may persist for a considerable time, in some cases as long as 30 days. Corroborative evidence was furnished later by the experiments of Issekutz (8) in which the results indicated that the digitalis bodies form a combination with the substance of the heart which breaks up with difficulty. The antagonistic effect of digitalis was especially well shown in the following experiments: Two minims of oil of chenopodium in 100 cc. Ringer's solution containing olive oil were perfused for 1 to 1½ minutes. Marked cardiac depression was produced and was increased when the test was repeated 9 minutes later. The same amount of chenopodium in suspension in the presence of tincture of digitalis produced a slight primary depression followed by pronounced stimulation lasting in one test 5 minutes. That digitalis not only neutralizes the effect of oil of chenopodium but may cause stimulation was repeatedly shown in tests made on the same heart.

The antagonistic action of digitalis could be demonstrated much better when higher concentrations of it were used, though the amount of oil of chenopodium was increased. Thus in one experiment the heart was perfused 4½ minutes with Ringer's solution containing 1 per cent. tincture of digitalis and 3 minims oil of chenopodium per 100 cc. No change could be observed for 4 minutes. A slight decrease in amplitude without noticeable alteration in rhythm now appeared. Within 15 to 20 seconds, however, marked slowing of rhythm amounting to 50 per cent., and increase in amplitude occurred which, in several minutes, was 20 per cent greater than before oil of chenopodium was administered. Perfusion with the same amount of oil of chenopodium for 3¾ minutes carried out 24 minutes previously produced entirely different effects. After 3¼ minutes perfusion, the heart became markedly dilated, the contractions rapidly grew feeble until they almost disappeared. This condition lasted 3 minutes when the heart began to recover slowly, reaching a maximum in 6 minutes. Although the amplitude was now about the same as before administration of oil of chenopodium the rate was 35 per cent less. In another experiment

a concentration of 1: 250 tincture digitalis was used for reviving the heart arrested in diastole by oil of chenopodium. Half a minute after perfusion with digitalis was commenced, contractions appeared. They were weak at first but rapidly increased in force though appreciably smaller than before oil of chenopodium was administered. The rate, however, was not changed.

The presence of alcohol in the tincture of digitalis may also be taken into consideration. A number of tests were carried out, therefore, with alcohol and oil of chenopodium in Ringer's solution and olive oil. One-eighth to one per cent of alcohol perfused one to two minutes failed to produce any change in heart action. Oil of chenopodium, one minim per 100 cc. was likewise ineffective. The combined action, however, of one minim oil of chenopodium and one per cent alcohol produced moderate depression. The depression which digitalis had to overcome is probably greater, therefore, than that produced by oil of chenopodium alone.

EXPERIMENTS WITH CAFFEIN

Although caffein, when perfused in combination with oil of chenopodium, produced moderate stimulation in the fresh heart, this was not the case when the same test was performed later in the experiment. No antagonistic action could then be observed. The effect indicated that caffein failed to stimulate the heart since depression occurred as in tests with oil of chenopodium alone. The results in some experiments suggested, on the contrary, that it aided the action of the oil of chenopodium. This was especially the case in experiments in which 0.01 per cent caffein was perfused with oil of chenopodium. In one experiment only moderate decrease of heart action was caused by chenopodium alone, but when perfused with a solution containing caffein the heart stopped in diastole for nearly one minute (fig. 9). Similar results were obtained in another case. Experiments with Ringer's solution with the same amount of oil of chenopodium in suspension and 1:500 caffein showed but little difference from those already described, although in one case in which oil of chenopodium was followed by 1:500 caffein, slight increase in heart activity was observed, amplitude alone being affected. That caffein may also cause depression was shown in another experiment in which the heart had been previously perfused with oil of chenopodium (fig. 10). It may be recalled that a

similar observation was reported by Salant and Mitchell (9) on the isolated intestine, caffein likewise causing depression of smooth muscle previously subjected to the action of oil of chenopodium.

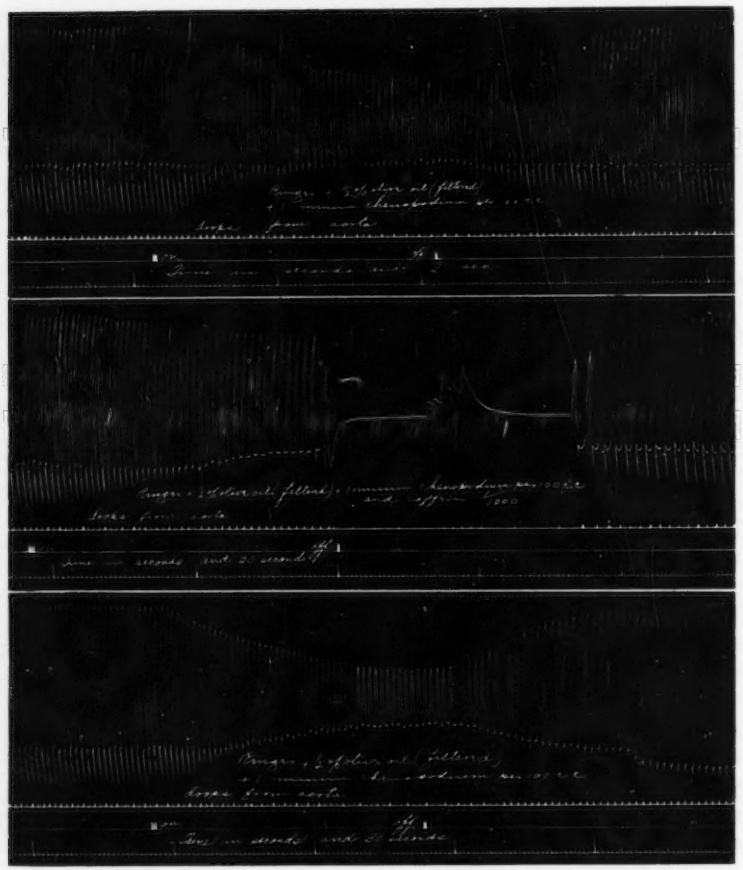


Fig. 9. *A*, Depression produced by oil of chenopodium. *B*, Combined effect of oil of chenopodium and 1/1,000 caffeine. *C*, The action of oil of chenopodium when perfused five minutes later.

Fig. 10. Frog 94. Cardiac depression produced by caffeine after the heart had been perfused with oil of chenopodium.

DISCUSSION

Although small amounts of oil of chenopodium either failed to cause disturbance of cardiac action in the fresh heart or the effect produced was very moderate, the result obtained indicated that it was a depressant and varied directly with the perfusion time and the amount present in solution. The increased cardiac depression observed in successive perfusions suggested cumulation at first but later this was considered doubtful. It was found that in some experiments the effect was independent of the number of tests with oil of chenopodium. A heart which had been perfused for some time reacted to oil of chenopodium as well as one which had been subjected to its action a number of times, thus indicating that the heart is rendered more sensitive to oil of chenopodium the longer perfusion with Ringer's solution or Ringer olive oil is continued. The cause of this condition has not been determined, but experimental evidence brought forward by several writers shows that it might be due to loss of substances essential to the normal action of the heart. According to Schücking (10) cardiac action can only be maintained as long as traces of blood remain on the surface of the cells, and when this is washed out the heart can no longer continue to function. Herlitzka (11) found organic acids in Ringer's solution perfused through the rabbit's heart. Clark (12) claimed that there was a loss of lipoids from the frog heart when perfused with Ringer's solution. He separated the product from the perfused fluid and found that it exerted a marked beneficial action upon the hypodynamic heart similar to that previously obtained by Danilewsky (13)

with lecithin. Jürss (14) has shown that thujone and fenchon dissolve lecithin. That it is also dissolved in oil of chenopodium was found in this laboratory. The lecithin and other lipoids of the heart might, therefore, be attacked by oil of chenopodium, thus producing the effect observed. The resistance of the fresh heart may also be thus explained. An abundant supply of lipoids in the intact animal may be assumed, the fresh heart containing a sufficient amount of them to stand the loss caused by the action of oil of chenopodium. As the store of lipoids is depleted by repeated perfusion, the action of oil of chenopodium would become more marked.

The increased effect of repeated perfusion and the difference in the behavior of the fresh heart and the heart after it has been perfused for some time may be due, therefore, to diminished amounts of lipoids present. The hypersensitiveness observed in some specimens may be similarly accounted for.

The mode of action of olive oil cannot be stated definitely. Inhibition of loss of lipoids by the oil or their accumulation at its surface causing cardiac stimulation as suggested by Clark (15) in the case of fatty acid is to be thought of as a possibility. But in view of the solubility of lipoids in olive oil, this explanation is not satisfactory. There would be no reason to expect the result to be different from that obtained with oil of chenopodium which is likewise soluble in lipoids. Its stimulating action on the heart probably depends upon other factors as yet unknown. The utilization of olive oil and the free fatty acids by the heart (thus replacing the lecithin which has been lost) is to be thought of in this connection. The formation of a protective envelope of colloidal particles around the cell, preventing the escape of lecithin, also suggests itself. The condition at the surface of the cell might become similar to that *in vitro* when lecithin, oils and soap are present. The stable emulsion formed would thus serve as a means of checking the loss of lipoids. The work of Lillie (16) is very suggestive in this connection. He has shown that the fatty acids penetrate the unfertilized egg of Arbacia and stimulate membrane formation and cleavage. As the same, in all probability, holds for the soaps, the cardiac stimulation observed with Ringer olive oil may be readily explained. The case is probably different with oil of chenopodium which resembles anesthetics in several respects. Thus, as already stated, we found that it aids the action of alcohol. Adrenalin restores cardiac action in paralysis caused by chloroform, chloral and oil of chenopodium. Lillie (17) observed that the action of neutral salts on

the eggs of Arbacia is inhibited by anesthetics which he believes caused decreased permeability of the cell. Perhaps the action of oil of chenopodium is due to a similar mechanism. The antagonism of oil of chenopodium and olive oil may be thus explained, although both are lipoid soluble substances. The action of adrenalin and digitalis to oil of chenopodium may also be correlated in some way with the action of the cell lipoids. That they may form some combination with lecithin appears from the work of Koch (18). According to Schliomensun (19) digitalis combines with alcoholic phosphatids which he isolated from the human heart and from that of the dog. The antagonistic action of adrenalin and digitalis to oil of chenopodium is also of interest in showing that the heart when thus poisoned may be improved by substances differing in their mode of action, which indicates that the injurious effect probably involves the intercardiac nervous mechanism as well as the muscular substance. This is in harmony with our previous observations on the action of oil of chenopodium.

The action of caffein when perfused in combination with or after oil of chenopodium furnishes additional evidence that the action of the drug may be reversed under changed conditions, which was pointed out in a previous communication by the writer (20).

An explanation of the depressing effect of caffein observed by us is suggested by the recent work of Secher (21). He found that caffein hastens muscle fatigue and may cause histological changes in the finer structure of voluntary muscle fiber even when a dilute solution is used. He also observed that this effect is not peculiar to caffein as chloroform may produce the same structural alterations in muscle fiber. In view of the similarity of action between oil of chenopodium and anesthetics these observations are very significant. Perfusion with caffein would be more effective in producing the structural changes and the increased cardiac depression caused by oil of chenopodium. Again, since the injury caused by oil of chenopodium may persist, subsequent perfusion with caffein would simply further increase the damage already produced. This would also explain the depression observed after caffein when the heart had been previously subjected to the action of oil of chenopodium. The synergistic or additive effect of caffein and of oil of chenopodium on the heart which we also observed in experiments on the isolated intestine make it highly probable that oil of chenopodium attacks the muscle fiber. This supposition is strengthened by the antagonistic effect of digitalis and oil of chenopodium.

SUMMARY

1. Oil of chenopodium produces marked depression of the isolated frog heart, the effect increasing with the concentration and successive perfusions. The fresh heart is less sensitive to oil of chenopodium than one which has been perfused for some time.

2. Olive oil added to Ringer's solution causes cardiac stimulation which may become more marked later in the experiment.

3. Oil of chenopodium is less active in the presence of olive oil.

4. Adrenalin and digitalis were found to be antagonistic to oil of chenopodium.

5. Caffein produced slight stimulation when perfused with oil of chenopodium but in some experiments it aided cardiac depression.

6. The action of oil of chenopodium may be due to disturbance of the lipoids of the heart. It is suggested that the antagonistic action of olive oil, digitalis and adrenalin may also be correlated with disturbance of lipoid metabolism in the heart.

7. Oil of chenopodium probably affects the muscular substance in the frog heart.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

I. The Electrical Response as an Index of Glandular Action[1]

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In the extensive literature of electrophysiology much has been reported concerning observed alterations of electrical potential in nerves and muscles under various circumstances, but relatively little attention has been given to the electromotive phenomena of glands. The studies of du Bois-Reymond, Rosenthal, Hermann, Luchsinger, Engelmann, and Biedermann on the cutaneous and other surface glands in the frog and fish and in the cat, dog, pig and goat indicated, more than thirty years ago, the possibilities of interesting research in this field. To some extent these possibilities have been realized in studies of the psychogalvanic reflex. The use of electrical methods for examining the glands of the interior of the body, however, has been almost wholly neglected. In 1885, Bayliss and Bradford (1) reported the results of an investigation of the electrical changes attending secretion by the salivary glands in the dog and cat, and a few years later Bradford (2) contributed further information on the same subject. In 1894, Bohlan (3) published a paper on the electrical effects produced by the gastric mucosa when stimulated through the vagus nerves. Any other papers dealing with electromotive phenomena of the glands that lie distant from the body surface, we have been unable to find.

An interest in the activities of the ductless glands, especially those without a secretion recognizable by present methods, led us to consider the possibility of applying to them the extremely delicate means now available for detecting slight electrical differences, with the hope that thereby further knowledge of their functions might be secured.

[1] The main results of this and the two following papers were presented before the American Physiological Society, December 29, 1915. See Proceedings, this journal, 1916, xl, p. 143.

Obviously an electrical change cannot yield information as to the nature of the materials elaborated in the several structures, it cannot determine the course which the products of secretion actually take in passing away from their region of origin, indeed it might leave somewhat uncertain whether secretion or preparation for secretion were the process occurring in a gland at any given period of disturbed potential. Nevertheless the method, by revealing the difference between relative quiet and activity, would indicate conditions which affect the functioning of glands and thus might suggest more definitive studies; it would show the quickness, the extent, and roughly the degree, of the response; and it might disclose significant new facts regarding relations which exist among the glands and which are mediated by the circulation. Despite its meager character such knowledge seemed worth seeking.

At the beginning of such a study of ductless glands there is obvious advantage in learning the capacities and limitations of the method as demonstrated in a gland which pours forth an easily recognizable secretion. For the purpose of preliminary tests we selected the submaxillary. And since we were, in the main, using cats in these researches we had the fortune of working on an animal in which both nerve supplies, the chorda and the sympathetic, evoke a considerable discharge of fluid. By use of the cat, therefore, it was possible to obtain insight into the electrical concomitants of a secretory activity which could be readily aroused from two different directions.

THE METHOD

The galvanometer used in these studies was of the d'Arsonval type.[2] Its sensitiveness is indicated by a deflection of 1 mm. on a scale 1 meter from the mirror when one volt is sent through 600 megohms. This sensitiveness, under the conditions of our observations, was sufficient for registering definite movements when physiological activity was induced in the glands, and yet was not so great as to make the instrument subject to minor adventitious alterations of potential.

The method of recording the galvanometer's deflection is shown by diagram in figure 1. On a firm wall-bracket, *T*, directly above the the scale, *S*, of the galvanometer, was placed a box painted black, inside and outside, and provided with a hinged cover, which when shut

[2] The instrument is that made by the Leeds and Northrup Company, Philadelphia, and listed as number 2220 in their catalogue.

admitted light only through a horizontal slit, *O*, about 1 mm. wide. This slit was 11.5 cm. above the top of the scale. Inside the box was the drum of a horizontal kymograph; and since the mechanism of the kymograph was outside the box the rate of revolution of the drum could be readily adjusted. When a record was to be made a sheet of sensitized paper was wrapped tightly around the drum and fastened with gummed paper, the drum was then set on the spindle of the kymograph and lowered into the box, and the box closed. All this process was carried on while the room was illuminated solely with ruby light. The light reflected from the mirror, *M*, to the slit in the box had its source in *E*, an electric bulb (6 volts, 21 candlepower) the filament of which was held vertical and permitted to send its rays out to the middle of the mirror through a narrow vertical opening. The rays reflected from the mirror were focussed on the sensitized paper[3] by means of a thin lens, *L*, placed near the galvanometer. The distance from the mirror to the paper was 45 cm. Obviously any motion of the mirror around the vertical axis of its support would result in a shift of the reflected beam of light along the horizontal slit, *O*.

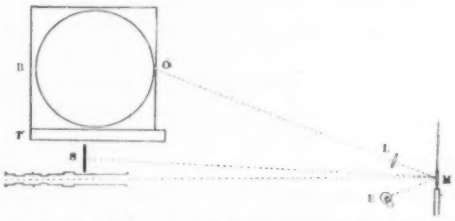

Fig. 1. Diagram of arrangement for observing and recording galvanometer deflections. For explanation, see text.

As indicated in figure 1, the arrangement allows the observer to watch the movement of the mirror through the telescope of the galvanometer at the same time that a record is being made.

A combined base line and time record was provided by a narrow beam of light directed on the slit, *O*, from another electric bulb. The electrical connections of this bulb are shown in diagram in figure 2. The current (at 8 volts) passing continuously through the filament of this bulb, *B*, was just sufficient to cause a slight glow which only dimly affected the sensitized paper. By means of another connection the dull glow of the filament was increased by an additional voltage, whenever a current was completed through the primary circuit of an inductorium *I*, by closure of the key, *M*. Thus the period of stimulation was registered on the revolving surface by a darkening of the dim

[3] The paper used was "standard" permanent bromide paper, quality B, made by the Eastman Kodak Company, Rochester, New York.

base line. A clock, making an electric contact every 30 seconds, momentarily magnetized the iron core, *C*, and thereby drew the spring, *S*, away from contact 1, and against contact 2 (these contacts were platinum). The stronger current thus sent for an instant through the filament produced a sharp flash which was registered as a black point on the base line. This registering of the half-minute intervals interfered only for a fraction of a second with any stimulation that might be in process.[4]

The non-polarizable electrodes used in contact with the tissues were similar to those described by Mines (4). Glass tubing, 8 cm. long and 5 mm. in internal diameter, was drawn to a point about 2 mm. in diameter. Into this pointed end was inserted a short length of soft cotton twine, which later afforded a gentle contact with the living tissue. The glass was then placed, point downward, in warm Ringer's solution containing 10 per cent gelatin. The fluid was deep enough to half fill the tubes. Six or eight such electrodes were thus prepared at one time. Usually this preparation was made in the afternoon for the next day. After standing over night in a cold room, the gelatin was so firm in the tubes that it supported in a thoroughly satisfactory manner the zinc sulphate poured in above it. When short zinc rods, soldered to copper wires[5] leading to the galvanometer, were placed in the zinc sulphate the electrodes were ready for use. These electrodes have each a resistance of approximately 1500 ohms. In the mass of gelatin in which they are set they can be kept for several days without deterioration, and removed as they are needed.

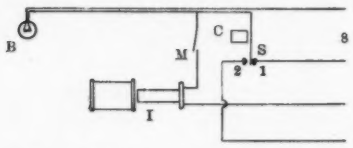

Fig. 2. Diagram of electrical connections for recording time intervals and duration of stimulation. For explanation see text.

Bayliss and Bradford, in their experiments on the submaxillary gland, set one leading-off electrode on the hilus of the gland and the other on the opposite surface. Apparently the object of this arrangement was to determine the difference of potential at the cell membranes lying between the duct and the lymph spaces. In our experience constant results have been obtained, however, with the gelatin electrodes situ-

[4] In developing the sensitive paper some sheets were darker than others. For uniform appearance the original photographs have been carefully copied in line drawings.

[5] The junction of the zinc and copper was covered with flexible wax.

ated one upon the gland being investigated, the other upon neighboring subcutaneous fascia[6] at a point near enough to other structures to be moistened with lymph. Secretion is then, except under conditions to be described later, accompanied by a negative relation of the gland to the indifferent surface, when these regions are connected through external conductors. Naturally the action current is occasioned only by such part of a circuit as is in fact active. And since stimulation of the chorda tympani, for example, makes active only the gland and not the surrounding structures, the current lines should be considered only with reference to the gland. The current lines would obviously be condensed under the electrode on the gland surface, but through the extensive area of contact between the gland and the tissues beneath it (which may be regarded as the other electrode) they would pass diffusely. In a complex gland like the submaxillary the highly confused relations of the cell membranes at which electrical differences would develop, and the simultaneity of action throughout the structure, would, in all probability, result in such antagonisms as to produce no effect whatever if only a sufficiently extensive region is taken for test. This suggestion is supported by the following experiment. We placed the two electrodes on outlying tissues on opposite sides of the gland and about 1 mm. distant from its border. Under these circumstances chorda stimulation failed to produce any action current that had an effect on the galvanometer; there appeared to be a nullification of the electrical differences in the secreting cells taken as a whole. An electrode placed at a point on the surface, however, should be regarded as having peculiar relations to the structures beneath. At that point doubtless there are cells facing ducts and backing on lymph spaces; and secretion by these cells would be accompanied by ionic alterations or by shifts of concentration, occurring at surfaces immediately below the electrode, which would definitely affect it. In other words, although a stimulated gland is simultaneously active in all parts, and consequently the attendant potential differences are likely to balance one another, still the electrical changes in a group of cells near the surface would be detected if one electrode is sharply localized in their neighborhood. These changes would produce current lines diverging from the electrode on the gland surface, passing widely-spread through the gland to surrounding tissues, and converging again to the electrode on the subcutaneous fascia. By this conception it has seemed to us possible to

[6] Subcutaneous fascia was selected as an indifferent tissue because of Waller's testimony to its inactivity.

account for the point on the gland surface being negative to the indifferent point on the skin. But whether this explanation is true or not, the fact remains.

One of the two wires connected with the zinc rods previously mentioned was marked with a black band. When the rod attached to this wire was placed in the gelatin electrode on the fascia, and the other rod was in the electrode touching the gland, a *negative* relation of the gland to the fascia through the external connections would cause the galvanometer to register on the sensitive paper an excursion *upward* (positive) from the base line. A *positive* relation was registered by a movement of the light beam in the opposite (*downward*, negative) direction.

As soon as the electrodes were in place on the tissues and connected with the galvanometer they invariably revealed a difference of potential between the two points of contact. This difference had to be compensated before the gland changes could be conveniently observed. In figure 3 is shown diagrammatically the arrangement of apparatus for compensation. The source of the opposed potential was a Daniell cell, *C*. Wires from the cell were connected with a pole changer, *P*, which permitted the direction of the current through the rheocord, *R*, to be reversed at will. A resistance box, *B*, interposed in the circuit, made readily possible any desirable adjustment in the resistance. And by means of the slide, *S*, which moved freely along the exposed wire of the rheocord, the demarcation current from the gelatin electrodes, *E* 1 and 2, could be precisely corrected.

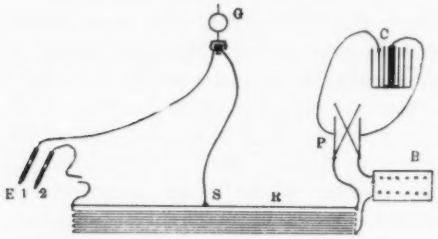

Fig. 3. Diagram of arrangement for connecting tissues with galvanometer, and for compensating the current of rest.

The presence of the compensating current, we found, became a source of error if the gelatin electrodes were both placed on the gland, or one on the gland and the other close by on adjoining tissue. Under these circumstances, after stimulation of the gland, by chorda impulses, for example, the galvanometer would indicate that the compensating current was prevailing; i.e., the galvanometer would move continuously outward in the direction which it would take if the compensating current were increased, or if the opposed current of rest were lessened.

An action current may or may not be demonstrated with both electrodes on the gland. When it is developed, it is probably due to a difference in the relations of the underlying active tissue to the two points of contact. If, with both electrodes on the gland, the action current has the same effect as the compensating current, the galvanometer moves out sharply in response to the glandular activity (action current), and instead of returning to zero, as normally, after the action current, it continues slowly outward. And if the action current is opposed to the compensation current, stimulation sends the galvanometer outward as usual and there is a prompt return to zero, but now the galvanometer continues to move slowly beyond zero, i.e., as if now controlled by the compensating current. This phenomenon can be accounted for by assuming conditions which lessen the current of rest (increased conductivity, increased permeability of membranes?). It does not occur when a sufficient amount of tissue *outside the active gland* intervenes between the electrodes. Then the factor of lessened potential of the current of rest is minimized by the inclusion in the circuit of considerable undisturbed tissue which offers a resistance little affected by changes in the gland, and the action current alone is manifested on excitation.

In certain cases there was a tendency for the galvanometer to move continuously in one direction even though compensation was repeatedly effected. Usually this phenomenon was more marked when the gland was first exposed than later. The reason for the phenomenon has not been determined; though probably one factor was a greater evaporation at one pole than at the other. When present it was a source of difficulty, for, dependent on the direction of the tendency to move, the return of the galvanometer to zero after an excursion was either incomplete or excessive.

In order that the galvanometer may give as true a record as possible of the electrical changes due to physiological processes it is necessary, of course, to keep the parts under and near the electrodes as quiet as possible. While working in the neck region we found that an L-shaped cannula inserted into the trachea, and clamped so that it held the structures in the larnygeal region in some tension, reduced to a minimum the influence of respiratory movements. Occasionally, exposed muscles in the field of operation will twitch. This trouble can usually be obviated by cutting across the muscles, or by severing their nerves. The various other ways in which motion at the contact between the electrode and the tissue was eliminated in different circumstances will be described later.

In some of the earlier experiments anæsthesia was dispensed with after the brain and the spinal cord (as far as the mid-thorax) had been pithed. Although such a preparation is quiet and is free from the influence of any depressive narcotic, the blood pressure is possibly too low for normal action. After some experience with urethane and ether, we came to the conclusion that etherization, if not too profound, was the best mode of rendering the animal motionless.

THE POSSIBLE FACTORS IN THE ELECTRICAL CHANGES IN GLANDS

Stimulation of the nerve connected with a gland may have at least two direct effects, one on the gland cells, the other on the smooth muscle of the blood vessels; and it may have at least two indirect effects, an alteration of the rate of blood flow through the vessels, and the motion of secreted fluid along ducts. Conceivably any of these variations might produce a change of potential. Evidently if vascular changes, vasoconstriction or vasodilation, were in themselves capable of giving rise to electrical differences, it would be difficult or impossible to draw conclusions regarding secretion in ductless glands having no demonstrable discharge. It is of prime importance, therefore, to determine what part any changes which are incidental to glandular activity itself may play in evoking electrical effects.

During the past five years evidence has appeared that contraction and relaxation of smooth muscle are accompanied by electromotive phenomena. The observations by Fuchs (6) on sipunculus and those of Brücke (7) on the retractor penis and of Arbeli and Brücke on the ureter have shown quite clearly that the electrical accompaniment of contraction is as characteristic of non-striate as it is of striate muscle. Still more pertinent was the observation by Straub (8), who noted, during the course of a study of the effects of certain conditions on the electrocardiogram, that the rise of blood pressure following asphyxia or a dose of adrenin was attended by a characteristic general shift of the galvanometer, which was recovered from as the pressure fell. He suggested that this phenomenon was a manifestation of the action current of the smooth muscle in the vessels. Although in Straub's experience local contraction of vessels, from exposure to cold, did not produce any electrical effect, and no shift was seen if the electrodes were on the same limb, the possibility of such an effect as a disturbing factor warrants careful consideration.

The question whether vasomotor changes might compound or modify

the electrical phenomena of glands was discussed and tested by Bayliss and Bradford, and later especially by Bradford (9). They made use of the effect of atropine in abolishing secretion without disturbing the action of nervous impulses on the smooth muscle of the blood vessels. After injecting atropine they found that when the chorda tympani failed to produce a salivary flow from the submaxillary gland there was simultaneously a disappearance of the electrical phenomena. Since atropine does not interfere with the vasodilation due to chorda influences the conclusion was justified that this vascular change was not a factor in the galvanometric response. Confirmation of Bayliss and Bradford's claim is seen in figure 4, which records electrical changes in the submaxillary gland on chorda stimulation (*a*), and on sympathetic stimulation (*b*), before the administration of atropine (1 cc. per kilo of a 1 per cent solution,injected intramuscularly between *b* and *c*); and the failure of the changes on chorda stimulation (*c*), five minutes after the injection, and on sympathetic stimulation (*d*) with the same weak current that was employed at first. When the current strength was increased sympathetic stimulation was again effective (at *e*), but no response could then be evoked through the chorda tympani.

There is the possibility that, although relaxation of smooth muscle in the vessels of a gland does not disturb the galvanometer, contraction might do so. Of course a shortening of the vascular muscle occurs during recovery from chorda dilation, and yet, as shown above (figure 4, *c*) no deviation of the galvanometer appears. The sympathetic influence on the vessels of the gland (in the dog) affords evidence, however which is more positive. Atropine in a sufficiently large dose not only inhibits the influence of the chorda tympani but also that of the sympathetic impulses. And Bradford found that when such a dose is given to a dog the abolition of the secretory effect of the sympathetic is coincident with the abolition of the electrical phenomena. Still other evidence that vasoconstriction alone, in the absence of secretion is not effective in producing electrical differences is found in the results of injecting pituitrin. As is well known, pituitrin causes a general rise of blood pressure by a direct constrictor action on the arterial walls. We have injected pituitrin[7] (0.2 cc.) into the femoral vein of a cat and raised the general blood pressure from 102 mm. of mercury to 158; there was, however, no secretion, and the galvanometer showed no electrical change whatever.

[7] We used the fluid preparation made by Parke, Davis and Company, Detroit.

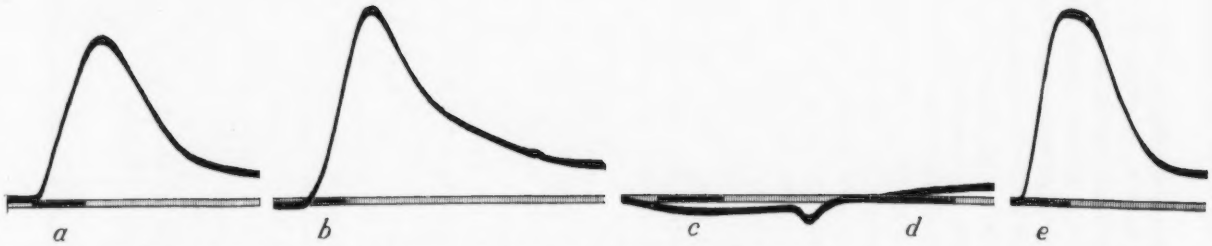

Fig. 4. Abolition of typical electrical response of submaxillary gland by atropine. Upper record, galvanometer; lower record, periods of stimulation; at *a* chorda stimulation 10 seconds, at *b* sympathetic stimulation 10 seconds, between *b* and *c* atropine 1 per cent, 1 cc. per k. injected intramuscularly, at *c* (5 minutes after the injection) chorda stimulation, at *d* sympathetic stimulation, at *e* sympathetic stimulation with much stronger current, which was ineffective when applied to the chorda. Time not recorded.

From these various experiments the conclusion seems well warranted that the electrical phenomena accompanying secretion are not due either to contraction or to relaxation of the smooth muscle in the vessels of the gland.

Another condition concomitant with vasomotor changes and also with external secretion is an altered rate of flow of fluid through capillaries and ducts. It has long been recognized that the movement of fluid through fine tubes is capable of causing differences of potential (10). May not the faster or slower blood flow and the progress of secreted material occasion the electrical phenomena?

Local vasodilation would be accompanied by a faster streaming, local vasoconstriction by a slower streaming through the capillaries. As already noted, both vasodilation and vasoconstriction will occur after atropine has stopped secretion of the submaxillary gland. But when secretion is lacking the electrical effect also is lacking. Under these circumstances, therefore, the altered rate of flow cannot have any influence in creating a difference of potential. We have tested the possibility further by raising blood pressure in the head through sudden occlusion of the aorta below the diaphragm and by suddenly lowering the pressure through compression of the heart. A pressure of 120 mm. of mercury was thus raised to 182 mm. and later lowered to 66 mm. Neither the faster flow from the high pressure nor the slower flow from the low pressure had any effect on the galvanometer. An altered rate of blood flow, we may conclude, is not a factor in producing the electrical change.

Although, in our experience, differences in the speed of streaming blood had no electrical effects, complete stoppage of the stream usually produced a deflection of the galvanometer which varied in extent and promptness of appearance. It seems probable, because of these variations, that the electrical changes are not consequent on the physical changes in the blood flow but are due rather to the influence of anemia on the gland cells.

Evidence indicates that the movement of secreted material along minute ducts of a gland likewise does not condition the electrical response. Bradford (11) noted, and we have confirmed him (see figure 5), that the galvanometer deflection starts before the saliva begins to move, and the maximum rate of the deflection is reached before the maximum rate of flow from the duct. Furthermore, when the escape of saliva is prevented by compressing the duct, repeated stimulations cause the usual electrical effect, though, as might be surmised from the

development of a back-pressure, the effect becomes gradually less (see figure 6, *b*, *c*, *d*). If now by removal of the compression the accumulated saliva is allowed to flow freely no potential difference appears (see figure 6 *e*).[8] This absence of an electrical change as secreted fluid begins to move or ceases to move along ducts is in accord with the conclusion reached in the previous paragraphs that the physical changes in the blood flow—starting, stopping, or going faster or slower—are not the occasion for electromotive phenomena.[9]

Fig. 5. Electrical change starting before saliva begins to move. Upper record, galvanometer. Lower record, dark band, chorda stimulation, 10 seconds; vertical lines, drops of saliva, except first one which marks moment when saliva began to flow in cannula. Last drop, white line on curve. Time not recorded.

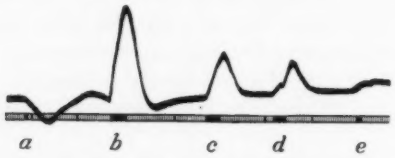

Fig. 6. Action currents of submaxillary gland after duct closed. Upper record, galvanometer. Lower record, time in half-minutes; at *a* clip on duct (manipulation); at *b*, *c*, and *d*, chorda stimulation, 10 seconds; at *e* clip removed (manipulation).

An action current will accompany secretion after the blood supply has been removed. Figure 7 records the electrical changes occurring in the right submaxillary gland. At *a* the right common carotid artery was clamped (the left had been clamped previously). About one minute later (at *b*) the cervical sympathetic was stimulated for ten seconds. The usual electrical change occurred attended by secretion.

[8] It was impossible to avoid slight electrical changes due to manipulation while the clip was being applied and removed (at *a* and *e*).

[9] In this discussion the reservation is made that a more sensitive galvanometer might reveal electrical differences not shown by the instrument used by us.

At *c* the clamp was removed from the right carotid. The record of electrical change differs from the normal chiefly in the absence of a rapid return to the base line (cf. figures 5 and 6), but the upward curve is characteristic and adds further to the proof that the electrical response does not arise from vascular changes. Favorable to that conclusion, also, is Bohlan's observation that vagus stimulation calls forth an action current of the gastric mucosa even though the aorta is clamped.

Other reasons for concluding that the functional activity of the secreting cells, and not muscular contraction or movement of fluid through fine tubules, determines the appearance of an action current in glands, will be noted in later papers. Thus the latent period of the action current in the thyroid gland differs from that of the adrenal, and in both these glands the period is much longer than in the submaxillary. Also the duration of the action current differs markedly in different glands; in the submaxillary it may last not more than a half-minute, in the thyroid and adrenal it commonly lasts much longer, as a result of brief electrical stimulation of nerves. There is no reason for supposing that the smooth muscle in the blood vessels of these glands varies either in the quickness of its response or in the persistence of its altered state (contraction or relaxation). The variations in the character of the action currents, typical of the several glands, contribute strongly to the argument that the electrical changes are reliable manifestations of a functional response.

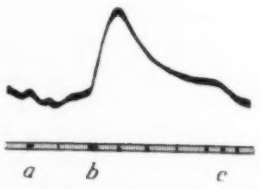

Fig. 7. Action current of submaxillary gland after carotid arteries closed. Upper record, galvanometer, connected with right submaxillary gland. Lower record, time in half-minutes; at *a* right carotid closed (left closed previously), at *b* sympathetic stimulated 10 seconds, at *c* clip removed from right carotid.

SUMMATION PHENOMENA

As illustrating the possibility of using the electrical method to obtain insight into the conditions of activity of glands the results of combined chemical and nervous stimulation may be cited. Figure 8 shows the usual effect of nervous influences after pilocarpine. At *a* 1 cc. of pilocarpine (1:1000) was injected into the femoral vein. A persistent secretion soon started, accompanied by a persistent positive electrical change. At *b* the chorda tympani was stimulated for 15 seconds, but

without effect. About 30 seconds later (at *c*) stimulation of the cervical sympathetic trunk sent the galvanometer deflection from +40 to +35, then out to +42, and slowly back to +25.[10] In this instance the excitation induced by pilocarpine was such that the chorda influences, which pilocarpine is supposed to mimic, no longer had any effect. The sympathetic influences, however, were still capable of increasing the activity of the gland.

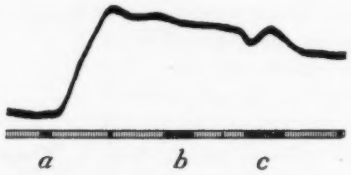

Fig. 8. Ineffectiveness of chorda impulses and effectiveness of sympathetic impulses during pilocarpine action. Upper record, galvanometer. Lower record, time in half-minutes; at *a* 1 cc. pilocarpine (1: 1000) injected intravenously, at *b* chorda stimulation, at *c* sympathetic stimulation (see text).

Fig. 9. Effectiveness of chorda impulses and ineffectiveness of sympathetic impulses during adrenalin action. Upper record, galvanometer. Lower record time in half-minutes; at *a* 1 cc. adrenalin (1: 10,000) injected intravenously, at *b* chorda stimulation, at *c* sympathetic stimulation, at *d* another adrenalin injection (1 cc., 1: 10,000), at *e* sympathetic stimulation, at *f* chorda stimulation.

The conditions of excitation in the submaxillary are quite different when adrenin is administered. In the cat this substance calls forth a secretion of watery saliva and an attendant electrical effect. In figure 9 is shown the record of the galvanometer after 1 cc. of adrenalin (1: 10,000) was injected (at *a*) intravenously. When the effect was maximal, the chorda tympani nerve was stimulated (at *b*), and the movement in the positive direction was definitely increased; at *d* an-

[10] The plus sign (+) indicates a movement of the galvanometer so that record *above* the base line is registered, i.e., the gland is negative to the fascia (see p. 44). The numbers refer to millimeter divisions on the galvanometer scale.

other injection of adrenalin was made, and near the summit of the curve stimulation of the sympathetic was without influence, but when on the return the chorda was stimulated a considerable increase of the positive deflection was registered (from +5 to +12). In another instance, after sympathetic impulses had been proved impotent during the acme of adrenalin action, a second dose of adrenalin caused an excursion to +26; and when at this maximum, chorda stimulation for 10 seconds increased it to +41. In these instances excitation of certain glandular components by adrenin made ineffective the sympathetic impulses which this substance simulates; but other components subject to chorda influences were not involved, and when they were stimulated, the gland, as the galvanometer shows, became still more active.

Confirmation of these results was obtained by stimulating for five seconds, first the chorda tympani, and then, after the galvanometer had moved to a maximum of +22, stimulating for the same period the sympathetic trunk. The excursion was thus increased to +32. Conversely, when sympathetic stimulation preceded and the deflection had reached +20, excitation of the chorda fibres increased it to +42.

REVERSAL OF THE SIGN OF THE RESPONSE

A phenomenon which has been repeatedly noted in electrical studies of glandular action is reversal of the response. Thus Engelmann (12) reported that the usual effect of stimulating the nerve of a piece of frog's skin is a negative variation of the normal "in-going" (i.e., external surface negative to internal through the galvanometer) current of the skin. In Hermann's observations (13), on the other hand, the commonest effect was a positive variation—an increase—of the normal current. Bayliss and Bradford (14), although admitting that it is "scarcely possible to speak of a normal excitatory variation," found, by making examinations throughout the year, that most of the time they confirmed Hermann but during the breeding season (February and March) they saw what Engelmann had seen. According to Waller (15) the reversal of response may occur within a few minutes—the first effect of stimulation is in the Engelmann sense, the second, five minutes later, is less so, and the third, five minutes later still, is in the direction Hermann described.

We have had similar experiences in recording the action currents of internal glands. For example in one animal the thyroid gland when stimulated will be negative in relation to the indifferent skin, in an-

other it will show regularly a positive relation.[11] This is admittedly disturbing. Various suggestions come to mind as possibly accounting for it, such as the position of the electrode on the gland, the degree of pressure, a reversal of activity, the lessening of an activity already existent rather than its increase on stimulation, or so much greater permeability that the compensating current prevails. Many of these suggestions were ruled out by a result which we obtained in some animals on stimulating either the chorda tympani or the cervical sympathetic trunk after the submaxillary gland was set in prolonged secretion by pilocarpine (1 cc. 1:1000, intravenously). Under these circumstances the nerve impulses, instead of causing the gland to become increasingly negative to the skin, rendered it less negative (see figure 10, *c*, *d* and *e*). And when the potential accompanying the secretory activity was compensated, sympathetic or chorda stimulation (figure 10 *f*) instead of making the gland negative to the skin (as figure 10, *a*) now made it positive. Later the nerve impulses caused a primary slight movement of the galvanometer in the "normal" direction, but there followed at once a much more extensive excursion opposed to that. In these experiments there was no shift in the position of the electrode nor any variation of its pressure on the gland. As a result of the stimulation there was the normal effect of chorda impulses—a marked increase in the rate of secretion. Lessening of the physiological process or reversal of it cannot, therefore, be invoked to account for the phenomenon.

For the present the reversal of the sign of the response remains unexplained. It may belong in the same category with numerous other instances of exactly opposed effects of the same stimulus, dependent on the state of the responding tissue (16). From the evidence here given, however, we may rest assured that the electrical change, either positive or negative, which follows stimulation is an indication of glandular activity. Like other indicators of fundamental physiological processes the electrical change develops gradually to a maximum and more gradually subsides again, and it follows after a definite latent period. As Waller has remarked, the obvious delay between excitation and response is "a sure and reassuring sign that we are dealing with a true physiological response, outside all possibility of coarse physical fallacy."

It is obvious that many more aspects of the functioning of the submaxillary gland are capable of investigation by the electrical method.

[11] The terms "negative" and "positive" we employ with the understanding that they refer to two regions as related through external connections.

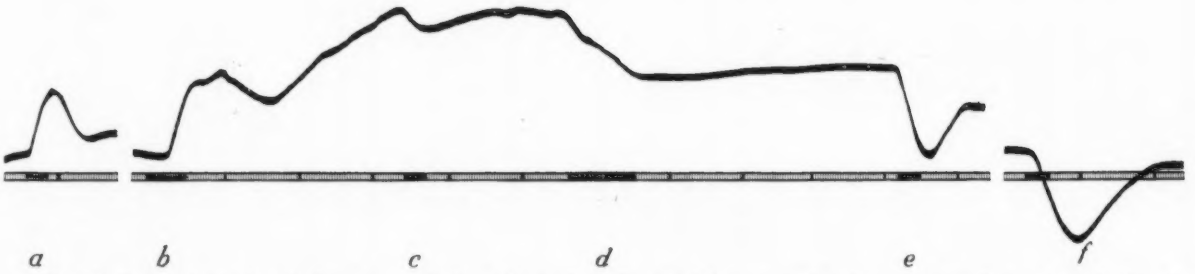

Fig. 10. Reversal of electrical response during pilocarpine effects. Upper record, galvanometer. Lower record, time in half-minutes; at *a* chorda stimulation, at *b* 1 cc. pilocarpine (1: 1000) injected, at *c* sympathetic stimulation, at *d* sympathetic stimulation again, at *e* chorda stimulation (with return to zero), at *f* chorda stimulation again after compensation. Secretion continued during the whole period after *b*, and was increased during the nerve stimulations.

For the present, however, they may be left. The brief study here recorded was of value to us chiefly for justifying the method as a means of getting more knowledge of the conditions of activity of ductless glands. We believe that the evidence here presented warrants the employment of this method in such an investigation and that when a difference of electrical potential appears in a gland as a result of an altered condition the electrical effect is substantial witness to secretory activity.

SUMMARY

A method is described for registering the excursions of a d'Arsonval galvanometer while permitting direct observations of the deflection on the scale.

Because (1) an electrical change accompanies secretion of saliva, even though the blood supply is shut off from the gland, or the flow through the duct is stopped; and because (2) the change is absent when secretion is absent although each of the conditions attendant on secretion—as contraction of blood vessels, relaxation of blood vessels, faster flow of blood, slower flow of blood—may severally be induced, the conclusion is drawn that the electrical change is a manifestation solely of the secretory process.

When the action current indicates a maximal activity of the submaxillary gland, excited by sympathetic impulses, the electrical response can be augmented by chorda impulses and *vice versa;* sympathetic impulses are ineffective during the height of an adrenin effect and chorda impulses cause no increase while pilocarpine is strongly operative.

The direction of the action current from the submaxillary gland may be reversed although the physiological response to stimulation remains as usual. Reversal is therefore not a sign of a reversed process in the gland.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCERINE GLANDS

II. The Secretory Innervation of the Thyroid Gland

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"One of the most difficult and yet one of the most important problems of modern physiology is the question of the innervation of the glands of internal secretion." With this sentence Osokin begins a recent paper on the thyroid gland. Among our chief objects in undertaking this series of studies on the electrical phenomena of glands was to throw as much light as possible into the obscure regions of our acquaintance with the innervation of the ductless glands. Because of an interest which one of us had in another research on the thyroid we selected this gland for our first examination.

Histological evidence. That the thyroid is well supplied with nerves has been known for many decades, as a result of histological studies. There has been general agreement among investigators that these nerves have not been distributed solely to blood vessels, but come into relation besides with the peculiar glandular cells of the organ. As far back as 1867, Parameschko (1) found nerves leaving the vessels and passing away as varicosed branches in the connective tissue between the follicles. In 1875 Poincaré (2) made similar observations, and intimated that these fibres might have a secretory function. And since then plexuses of nerve fibres about the follicles and connected with the bases of cells have been reported by Crisafulli (3), by Andersson (4), and by Berkeley (5). Andersson's description has recently been confirmed by Rhinehart (6). According to this latest work all nerves entering the thyroid gland are non-medullated; they arise from neighboring cervical sympathetic ganglia, accompany the arteries, and follow their branches after entering the gland. From the perivascular plexuses fibres are given off to interfollicular connective tissue and form there other plexuses completely surrounding the follicles. From

each perifollicular plexus the finest fibrils reach out and end in knob-like enlargements on the basal end of the gland cells.

From the analogy of other glands provided with secretory nerves, we should expect that the cells of the thyroid would be supplied solely by fibres from the autonomic nervous system. The histological evidence, just cited, that only non-medullated fibres enter the gland, gives confirmation to that surmise. These fibres, however, might be derived from both the sympathetic and the cranial divisions of the autonomic system, or from either division alone. It is a matter of considerable significance for understanding the relation of the thyroid to the bodily economy to learn their origin.

Anatomical evidence. After careful anatomical and embryological dissections Briau (7) reported that the thyroid nerves arise from the cervical sympathetic trunk, leaving that trunk at different levels, but originating especially (in man) from the middle cervical ganglion. This mass of nerve cells usually lies upon, or close to, the inferior thyroid artery, hence the name "thyroid ganglion" assigned to it by Haller. It corresponds to the inferior cervical ganglion of the dog and cat. Even what in man has been described as the inferior cervical ganglion is frequently found fused with the first thoracic. It is this fusion of several ganglionic masses at the top of the thorax that makes the stellate.

In man the fibres passing out from the middle (inferior) cervical ganglion form plexuses around the thyroid arteries, Briau states, but chiefly around the inferior artery. According to Poirier and Charpy (8) and other anatomists, branches from the superior cervical ganglion make the superior thyroid plexus about the artery of that name, and penetrate the gland along with it. Henle's observation that the superior laryngeal nerve, and Lindemann's observation that the recurrent laryngeal each sends two or three filaments to the gland, were confirmed by Briau in all his dissections.

In the cat the inferior cervical ganglion lies low in the neck, and the superior cervical is situated well above the level of the thyroid cartilage. The inferior thyroid artery along which in man filaments from the inferior ganglion pass to the gland is inconstant in the cat, and even when present is small. The superior thyroid artery is a regular branch of the common carotid. Langley states (9) that occasionally in the cat a small "accessory" ganglion is found on the cervical sympathetic trunk, about the level of the superior thyroid artery, which sends off gray fibres to that artery. In most animals this ganglion is part of the superior cervical.

Evidence from stimulation or severance of nerves. Further indication of the character and relations of these nerves is found in the experimental work done to determine whether or not they have a secretory function. Evidence has been obtained by stimulating the nerves or severing them, and testing the effects on other functions or on the condition of the gland itself.

In the Bern laboratory Asher and his collaborators have used the method of stimulation and examination of other functions. They have followed a suggestion made by Cyon (10) that the superior and recurrent laryngeals are the secretory nerves for the thyroid. When these nerves were excited Asher and Flack (11) found that the depressor causes a greater fall of arterial pressure and that adrenin raises arterial pressure to a greater height than before. Because the same phenomena occur after intravenous injection of thyroid extract they concluded that the laryngeal impulses call forth a true secretion. Asher and v. Rodt (12) reported that stimulation of the laryngeals increases the efficacy of splanchnic impulses. And Osokin (13) observed that such stimulation magnifies the influence of the vagus on the heart. Although Biedl and Brun (14) were able to demonstrate that the depressor causes a greater fall of pressure after the laryngeal nerves have been excited than before, they were unwilling to concede that this result was a reliable indicator of thyroid secretion, because in the same experiment uniform stimuli applied to the depressor resulted in a variable fall. And Schäfer has testified (15) that he has been unable to confirm, either in the rabbit, the dog, or the cat, the claim that injection of thyroid extract increases the efficacy of the cardiac vagus or the depressor. In any case, whether Asher's criterion of thyroid discharge is trustworthy or not, the method leaves undecided the interesting question whether the impulses are delivered by cranial autonomic or sympathetic neurones. For although the laryngei are branches of the vagus and therefore contain, presumably, elements of the cranial division, they might, on the other hand, serve merely as conveyors of sympathetic fibres.

Somewhat more direct evidence as to the cranial or thoracic origin of the thyroid nerves is found in tests of the iodine content of the gland with and without stimulation. Examinations by Wiener (16), by Rahe, Rogers, Fawcett and Beebe (17), and by Watts (18) agree in showing that the iodine content per gram of gland substance, though varying in different animals, is practically identical in the two lobes of any single animal. Rahe and her collaborators discovered that stimu-

lation of the superior thyroid vessels with the accompanying nerves, or the vago-sympathetic trunk (in the dog), may make the iodine content of the disturbed lobe less than that on the other side by as much as 0.3588 mgm. per gram of gland substance. They concluded, therefore, that a discharge of the peculiar substance of the thyroid takes place in response to nervous stimulation. This conclusion has been supported by Watts (19) who, after stimulating the cervical sympathetic trunk, or the nerve filaments accompanying the superior thyroid artery (in the dog), found a considerable reduction of the iodine content of the thyroid on the stimulated side. After stimulation of the entire sympathetic trunk the average reduction (0.04572 per cent for dry tissue) was more than it was when the thyroid filaments alone were excited (0.03526 per cent).

Additional evidence indicative of sympathetic influence over thyroid activity was obtained by Missiroli and by Wiener. Missiroli (20) found at various periods after section of the sympathetic trunk in the neck characteristic changes in the granules of the secreting cells of the thyroid. Wiener (21) studied the effects of cutting not only the sympathetic, but also other nerves. He severed the vagus on one side (in the dog) above the ganglion nodosum, but without effect—the glands on the two sides corresponded in weight and in iodine content just as in normal animals. The result was quite different after removing the inferior cervical ganglion; within a few weeks a remarkable diminution in size occurred on the operated side (in three cases a lessening of dry weight of 36.9, 48.5, and 11 per cent),[1] and with that a corresponding reduction of the thyroglobulin content. Katzenstein's earlier observation (22) that severance of the superior laryngeal nerve results in a degeneration of cells in the gland, Wiener explains as a consequence of section of sympathetic fibres in the superior laryngeal, because cutting the main trunk of the vagus is without effect.

He concluded that if paralysis of the sympathetic causes atrophy and a lessened iodine content, stimulation must cause hypertrophy and increase of thyroglobulin—a conclusion which is opposed, however, by the results of the stimulation experiments already described.

The various interpretations which have been given to the experiments above sketched will be left to later discussion. For the present it is sufficient to state, in summary, that the evidences from histologi-

[1] In a cat with the thoracic sympathetic cord cut just below the stellate ganglion we found after four months the thyroid lobe on that side weighing 0.167 gram, that on the other side 0.256 gram.

cal and anatomical examination, and from experimental excitation and severance of nerves, point towards a definite influence of sympathetic impulses over thyroid activity.

Electrical evidence for the innervation of the thyroid gland.[2] In a previous paper (23) we have given reasons for believing that the action current of a gland is a reliable indication of secretory activity. The two phenomena, as tested in a gland with external secretion, appear together, vary together in duration, disappear together, and are independent of accompanying vascular disturbances. This close concomitance of secretion and electrical change justifies the use of the electromotive phenomena of a gland to determine the conditions of its activity.

Methods. The general methods which we have employed in this research we have described in the first paper of this series. We found in studying the thyroid gland that urethane (2 grams per kilo) was unsatisfactory as an anaesthetic, and also that deep etherization was capable of abolishing effects that appeared readily when the anaesthesia was not so profound. In some experiments we obtained consistent results on dispensing with any anaesthetic after pithing the brain and the cord to the mid thorax. Since the blood flow was thus much diminished, however, we decided that etherization to the point of immobilizing the animal was a less upsetting procedure, and consequently we have employed that method in most of our work. In a few cases we have used curare after decerebration.

Through an opening between the second and third ribs the right sympathetic trunk was laid bare. Shielded electrodes which could be fixed in place[3] were slipped under the nerves just below the stellate ganglion, and the conductivity of the fibres below the electrodes was destroyed by crushing. The skin was now drawn close around the

[2] The brief description of experiments which follows gives no indication of the troubles which were encountered in the course of our investigations. The method was new to us, it was new in its application to glands of internal secretion, and was beset not only with experimental difficulties, but also with dangers of serio s error. We lost much time before we learned that deep anaesthesia would check secretion. We had difficulties in securing immobility and excluding the effects of action in neighboring tissues. We also had troubles with electrodes and with diffuse action of chemical stimuli. All these and other conditions we were compelled to examine for their influence on the processes being studied and on the methods which we were using. After we had learned the pitfalls, however, and could intelligently avoid them, we found it possible to demonstrate in one preparation practically all the points here described.

[3] See Cannon and Nice: This journal, 1913, xxxii, p. 47. A small spear-shaped steel hook reaching out of the wood held the rod in any position desired.

projecting part of the electrodes. Artificial respiration was maintained by an interrupted air current. The leading off (gelatin) electrodes, connected with the galvanometer, were set, one in contact with the right thyroid gland which had been exposed with as little manipulation as possible, the other on the connective tissue inside of a little flap of skin turned back from the neck at about the thyroid level, or at times on the trachea. Movement of the parts near these electrodes, due to respiration, was practically abolished by clamping the tracheal cannula and pulling it slightly towards the chest. Usually just after the gland was exposed and the gelatin electrodes were applied a period of repeated adjustment had to be passed through before the tissues and the compensating current were in equilibrium.

Effects of sympathetic stimulation. Numerous observations have shown that when the sympathetic trunk immediately below the stellate ganglion is stimulated by a weak tetanizing current a definite difference of potential is developed between the two leading-off electrodes. This phenomenon does not occur if the stimulus is applied to other nerves in the upper thorax, e.g., the central end of the cut phrenic. It is not, therefore, due to any spreading of direct physical influences from the thorax to the upper neck region. Furthermore it does not occur if the leading-off electrode on the gland is transferred to a neighboring muscle, and the thoracic trunk is then stimulated. It is, therefore, due to some change in the thyroid itself. That the electrical change has a characteristic and long latent period—usually between 5 and 7 seconds after the stimulus is started—which is considerably longer than that for the submaxillary gland, confirms the other evidence that the change is not direct physical effect, but a peculiar and distinguishing manifestation of thyroid activity.

In the first paper of this series, on the submaxillary gland, records were published illustrating the gland negative to the skin (through external connections) as a result of nerve stimulation, and also, but under different circumstances, the gland positive to the skin as a result of the same stimulation. The reversal of skin currents, also, in consequence of repeated nervous excitations during a short period, was referred to. Reversal of the current is not yet explained, but that it does not invalidate the electrical phenomenon as a criterion of activity is proved by the observation that a typically increased flow of saliva follows chorda stimulation, whether the gland becomes negative or positive to the skin (24). These facts are recited here because, in our experience, the thyroid gland, although usually negative to the skin

after stimulation, is sometimes positive for a moment and then negative. More rarely a purely positive relation to the skin is present.

Illustrations of these three types of responses are given in figure 1, *A* and *B*, and in figure 2. Other records taken before and after these in the same animals had the same peculiarities. The latent period in the case of figure 1 *B* was longer than in the case of *A*, a condition which was noted in several other instances in which the reaction occurred in

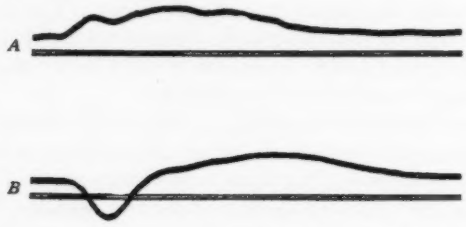

Fig. 1. Action current of the thyroid gland on stimulating thoracic sympathetic cord. Here and in other records time is registered in half-minutes. *A*, stimulation one minute; gland negative to subcutaneous fascia. *B*, stimulation a half-minute; gland first positive, then negative.

Fig. 2. *A*, 2.39. Thyroid action current from stimulating for a half-minute the thoracic strand, with all nerves intact. Gland positive to trachea. *B*, 2.43. The same, except recurrent laryngeal nerve cut (2.41). *C*, 3.09. The same, except, in addition, superior laryngeal nerve cut (2.48). *D*, 3.21. The same, except, in addition, cervical sympathetic cut above inferior cervical ganglion (3.21).

two phases. The greater delay under these circumstances may be due to an initial balance between two opposed processes; one of these processes may then hold primary predominance, only later to give way to the other.

The course of the sympathetic fibres. The action current evoked by stimulating the sympathetic nerve in the chest persists although the recurrent laryngeal nerve is severed. Figure 2 *A* presents the record of electrical change in the thyroid gland when the sympathetic strand

was stimulated (at 2.39) with all the cervical nerves intact; and figure 2 *B*, a record from the same animal (at 2.43) after the recurrent laryngeal was cut The conditions of stimulation were the same in both instances. The response was that of a positive relation of the gland to the indifferent tracheal cartilage. The operation has evidently not affected the influence of impulses started in the sympathetic fibres of the chest.

The action current of the thyroid persists also after the influence of the superior laryngeal nerve has been abolished. In figure 2 *C* the action current of the thyroid is registered on stimulation of the sympathetic trunk in the thorax (at 3.09) after severance of the superior in addition to the recurrent laryngeal. The stimulus was applied for a half-minute just as before; the larger response is probably not significant of a favorable effect of the operation, because, as proved by other instances in which the same experiment was performed, the response after the nerve cutting may be slightly less than before.

A further step in determining the path of the impulses which cause the electrical change is taken when the cervical sympathetic fibres are sectioned, and the thoracic trunk is stimulated. Figure 2 *D* is a photograph of the electrical change of the thyroid in the same case from which the other records in figure 2 (*A*, *B* and *C*) were taken, but after the cervical sympathetic trunk in addition to the laryngeal nerves had been cut across. Obviously these operations have not interfered with the reaction of the gland. Although the reaction continues after the sympathetic fibres have been transected in the neck, excitation of these fibres central to the cut produces the characteristic effect.

The question now arises as to where the relay between preganglionic and postganglionic fibres is situated. As already seen, two ganglionic masses are present in the course of the cat's cervical sympathetic—the inferior and the superior, with occasionally an "accessory" mass near the superior thyroid artery. That all the fibres leading to the gland do not originate in the superior ganglion or ganglia is proved by figure 2 *D*, for in that instance the sympathetic trunk had been cut across between the two cervical ganglia. It can be assumed therefore that in the cat a relay of some of the fibres occurs in the inferior ganglion.

Evidence for a relay in the superior region was obtained by the nicotine method of blocking impulses at sympathetic synapses and also by serial sectioning of the nerve from above downwards. In one of the experiments in which nicotine was used stimulation of the cervical sympathetic sent the galvanometer from +2 to −3, and thence out

to +94. A pledget of cotton wet with 1 per cent nicotine was now laid in contact with the superior cervical ganglion. Eighteen minutes later the nictitating membrane was not withdrawn when the sympathetic trunk was stimulated, but the galvanometer was sent out to +76; eight minutes later another stimulation for a half-minute sent it to +78. Now a slender strand of cotton wet with the nicotine solution was laid along the sympathetic trunk from the superior cervical ganglion to a point 2 cm., below the thyroid cartilage. After 20 minutes, stimulation of the cervical trunk had no effect. That this was not due to any deleterious effect of nicotine in the gland was proved by exciting the gland to activity by chemical stimulation (in a manner to be described later), and also by applying the electrodes to fibres on the superior thyroid artery and finding that repeated stimulation sent the galvanometer in the plus direction. In another experiment in which the sympathetic was serially cut, first across the upper pole of the superior cervical ganglion, second across the middle, and then crushed directly below the ganglion, stimulation of the cervical sympathetic trunk still persisted in causing marked action currents in the thyroid gland. The sympathetic trunk was now severed about 1 cm. below the ganglion, and thereupon the electrical effect of stimulation disappeared. These experiments indicate that in these animals the upper relay region was not situated in the superior cervical ganglion itself, for when impulses to the eye had been blocked in that ganglion by nicotine or the fibres had been cut below it, the electrical change in the thyroid still followed excitation of the sympathetic. The impulses were shut off, however, when the nerve was cut some distance below the ganglion or when nicotine was allowed to act on that region, i.e., the region in which Langley found the "accessory" ganglionic cells with a distribution of fibres to the thyroid. In another instance, after nicotine, applied to the superior cervical ganglion, had failed to prevent the thyroid action current (although effectively blocking nervous influence on the eye), a strong carbolic acid solution touched on the superior thyroid artery, in order to destroy nervous conductivity, quickly abolished the phenomenon.

After the cervical sympathetic trunk has been cut above the inferior cervical ganglion and a record of the thyroid action current has been obtained by stimulating the thoracic sympathetic cord (as in figure 2, *D*), excision of the inferior cervical ganglion abolishes the effects of stimulation.

From the foregoing evidence we may conclude that both in the superior and in the inferior ganglionic masses of the cervical sympathetic,

impulses to the thyroid gland pass from preganglionic fibres to the outlying neurones.

The question of vagus control. The prominence of laryngeal stimulation in previous experiments, especially the experiments carried on in the Bern Laboratory, raises the question as to whether the thyroid gland like the submaxillary of the cat, is subject to control by cranial as well as by sympathetic autonomic fibres. To secure evidence on this question, we had to stimulate the vagus trunk before its union with the cervical sympathetic strand. And since the vagus contains motor fibres to muscles of the larynx, which might through their contractions affect the galvanometer, it was necessary to use a curarized preparation. We decerebrated, and then, in order to avoid as far as possible the danger of disturbing the gland cells, injected intravenously barely enough curare to abolish reflexes. We bared the vagus at its point of emergence from the skull, tied a thread about it there, cut proximal to the thread and applied the stimulus to the nerve as close as possible to the thread, i.e., as far as possible from the union of the vagus and sympathetic at the superior cervical ganglion. We have not succeeded in obtaining any evidence of any influence of vagus impulses on the thyroid gland. We have stimulated the nerve with the gland at rest, and with the action current developing as a result of sympathetic impulses, and with the action current disappearing—but no change was produced. Figure 3 *A* is a record of the action current of the right thyroid as a result of stimulating for a half-minute the right thoracic sympathetic cord (at *a*). After a latent period of about 25 seconds (longer than with ether—probably due to curare and a lowered body temperature), the galvanometer began to indicate the gland negative to the indifferent tissue. When the galvanometer had moved to +12, the right vagus trunk was stimulated for a half-minute with the same stimulus (at *b*). The movement continued without check to +45, and was followed by a slow return. That vagus stimulation had no noteworthy influence, is shown by comparing figure 3 *A* with figure 3 *B*, a record taken three minutes after figure 3 *A*, and registering the

Fig. 3. *A*, 4.27. Thyroid action current on stimulating for a half-minute the thoracic cord at *a*, and the main vagus trunk at *b*. *B*, 4.31. The same, on stimulating the thoracic cord alone at *a*.

action current when the thoracic cord alone was excited for a half-minute (at *a*). The close resemblance of the two records proves that the vagus has been neither an exciter nor an inhibitor of thyroid activity.

From this evidence we may conclude that the laryngeal branches of the vagus nerve are not secretory nerves of the thyroid gland. And the fact that action currents of undiminished amplitude follow sympathetic stimulation after these nerves are severed (see figure 2) indicates that they are not highly important vehicles for sympathetic fibres.

Pharmacological evidence. A way of securing further evidence bearing on the source of impulses affecting the thyroid is found in the use of drugs having specific effects on cranial autonomic nerve endings. It is well known that pilocarpine stimulates viscera innervated by cranial autonomic nerves and affects these organs in such manner as to mimic the action of nerve impulses. In the case of the submaxillary gland, for instance, chorda impulses are commonly ineffective if the gland is already responding to an injection of pilocarpine (25). By use of pilocarpine, therefore, it is possible to obtain deeper insight into the nature of the nerve supply to the thyroid.

Figure 4 shows the lack of effect on the thyroid gland when 1 cc. of pilocarpine, 1: 1000, was injected (at *a*) intravenously. This is a dose which in the cat produces an abundant and long-continued salivary secretion, accompanied, as shown in the first paper of this series, by a persistent electrical change. But in the thyroid gland pilocarpine does not induce an action current—under no circumstances have we observed consistent indications of an effect on the thyroid which could be ascribed to this drug. These results are in accord with the failure of vagus impulses to influence thyroid activity; they support the conclusions from the histological studies by Schmid (26) and by Osokin (27), and the chemical studies by Wiener (28) that pilocarpine is wholly without effect on the thyroid; and they are further evidence against the opinions of Wyss (29) and of Andersson (30), based on microscopic appearances, that pilocarpine produces changes in the thyroid similar to those seen in a secreting gland. The weight of evidence, therefore, is opposed to a cranial autonomic supply to the thyroid gland.

The influence of anemia on thyroid activity. In a recent paper Watts (31) has reported that diminishing by mechanical means the blood supply to one thyroid lobe, periodically (for 2 seconds every 10) during approximately three hours, lessens the iodine content of that lobe relative to the other. The discrepancy between the two lobes was not so great as when a stimulus was applied to the cervical sympathetic or

to the fibres on the superior thyroid artery, but such stimulation, it was pointed out, would cause a greater check to the blood flow than could be caused by mechanical compression of the arteries. Watts and Carlson conclude, therefore, that shutting down of the blood supply by vascular constriction accounts for all changes in the thyroid thus far shown to be caused by stimulation of nerves.

We have put to test the idea that anemia accounts for thyroid secretion. In figures 1 *B*, 2 and 3 the period of stimulation was only a half-minute. Even if the result were a total stoppage of the flow through the gland, it would not persist after stimulation ceased. That complete anemia of the thyroid for a half-minute is practically without effect is shown in figure 5. For two periods, at *a* and at *b*, the blood

Fig. 4. Absence of thyroid action current on injecting pilocarpine (1 cc., 1: 1000) at *a*.

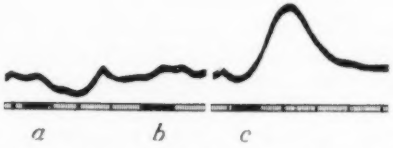

Fig. 5. Galvanometric changes in the same animal when thyroid completely anemic at *a* and at *b* (a half-minute each) and when stimulated (thoracic cord) a half-minute, at *c*.

supply to the gland was entirely excluded. This was done by first tying the left carotid artery low in the neck, and then, at *a* and at *b*, occluding the right carotid. It was impossible to avoid a slight amount of disturbance from manipulation, which would account for the uneven line of record. The effect of this procedure is to be compared with the effect of stimulating for a half-minute the thoracic sympathetic cord in the same animal, at *c*. Obviously nerve stimulation has an influence on the thyroid very different from anemia.

Discussion of the evidence for thyroid innervation. The observations presented in the foregoing pages are concerned mainly with three questions: (1) the nature of the innervation of the thyroid gland, (2) the

mode of action of the nerve impulses whether directly secretory or not, and (3) the character of the process underlying the electrical change.

We believe that the evidence is now convincing that the nerves of the thyroid are exclusively derived from the sympathetic division of the autonomic system. The histologists find non-medullated fibres reaching to the base of the follicular cells; the anatomists distinguish only cervical sympathetic nerves entering the gland; sympathetic section causes marked atrophy of the gland structure whereas vagus section has no influence; sympathetic stimulation gives rise to a striking action current, whereas excitation of the main vagus trunk, and exhibition of pilocarpine as a cranial autonomic stimulant are without effect. All these facts are harmonious in bearing testimony to an exclusive sympathetic control of thyroid activity. The observations on the effects of exciting the laryngeal nerves, made by other investigators, are not thereby rendered invalid, but the interpretation placed upon them is that whatever influence these nerves may have on thyroid function is exerted by way of sympathetic fibres contained in them—a possibility which, as we have seen (p. 59), has anatomical justification.

The question as to whether the nerves supplied to the thyroid are truly secretory in function has been raised for various reasons. Although regarding the sympathetic as the trophic and secretory nerve of the thyroid, Wiener has argued (32) that if section of the sympathetic produces atrophy of the thyroid, stimulation must produce hypertrophy, and accumulation of thyroglobulin. This conclusion, however, is not justified. Section of the chorda tympani, for example, results in a gradual lessening of the size of the submaxillary gland (33), and yet the normal function of the nerve is to cause a secretory discharge. Also Wiener's argument is opposed by the discovery that stimulation of the cervical sympathetic trunk actually lessens the iodine content of the gland. The view of Watts and Carlson that sympathetic impulses lessen the iodine content by producing anemia fails to regard both the direct nerve supply to the gland cells and also the reasonable assumption that these fibres have a function apart from the fibres distributed to blood vessels; and furthermore their view is opposed by the evidence presented in this paper that although sympathetic stimulation induces a marked action current in the thyroid, total anemia for an equivalent period brings forth no noteworthy electrical change whatever. Prolonged anemia may indeed lessen the iodine content of the thyroid, but tissues respond to a variety of stimuli; and merely because, for example, uremia causes convulsions, it is not proper to attribute all

convulsions to uremia. The conclusions of Watts and Carlson, therefore, seems to us defective in failing to regard important conditions which affect response. Still another doubt regarding secretory fibres has recently been raised by Manley and Marine (34) who found that when thyroid tissue is transplanted to various parts of the body it grows where placed, and functions and reacts as a normal gland would do. There is no logical opposition to secretory nerves in these experiments. The adrenal glands, for example, will serve the routine needs of the body when their secretory nerves are cut, for the animal lives with the nerves transected, but he promptly dies if the adrenals are removed. The capacity of an organ to give service, as the heart may do, though separated from the central nervous system, should not lead to the conclusion that its nerves are without influence. This doubt of the existence of specific secretory nerves to the thyroid has not, therefore, any convincing significance. And like the other suspicions as to the reality of these nerves, this one is based, as we see it, on a failure to consider all the evidence.

A part of the evidence for secretory nerves is, to be sure, first presented in this paper. But it is based on the assumption that the action current is a sign of secretory activity—and *that* might be doubted. To some degree the assumption is based on analogy with the submaxillary gland. An action current in that gland is the regular concomitant of secretion, and fails when secretion fails. The action current of the thyroid has a latent period characteristic of physiological processes, it may outlast the period of stimulation by many minutes, it appears after stimulation of sympathetic fibres—the filaments of which are known to be distributed to the gland cells—and all these evidences indicate that the action current is a true signal of physiological events taking place in the gland as a result of nerve stimulation. The concordant testimony of different investigators that precisely the sort of stimulation which provokes this process, will, if continued, result in a measurable diminution of the iodine content of the disturbed lobe affords clear proof that the process signalled by the galvanometer is one of secretory discharge.

SUMMARY

Histologists describe non-medullated nerve fibrils reaching to the cells of the thyroid gland. Anatomists find that fibres going to the thyroid gland arise in cervical sympathetic ganglia.

Previous investigators have shown that severance of its cervical sympathetic nerves causes atrophy of the thyroid, and that stimulation of these nerves causes a diminished iodine content of the gland. Severance of the vagus supply has no effect.

If the thyroid gland and neighboring indifferent tissue are connected through a galvanometer, stimulation of the sympathetic cord high in the thorax evokes an action current after a latent period varying usually between five and seven seconds. This effect persists after the superior and the recurrent laryngeal nerves are severed. Evidence is presented that the impulses pass to outlying neurones whose cell bodies are located close below the superior cervical ganglion and also in the inferior cervical region.

Stimulation of the main trunk of the vagus in a curarized animal, and injection of pilocarpine (as an exciter of cranial autonomic endings) have no effects on the thyroid gland.

Anemia made total by clamping the carotids for a period equal to that of sympathetic stimulation produces no noteworthy electrical changes in the gland.

The conclusion is drawn therefore that the non-medullated nerves distributed to thyroid cells belong to the sympathetic and not to the cranial division of the autonomic system, that their effects are not indirect through alterations of blood supply, that they are indeed true secretory nerves.

The arguments against this conclusion are discussed.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

III. The Influence of the Adrenal Secretion on the Thyroid

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One of the most interesting possibilities offered by the use of the action current as a sign of glandular functioning is that of testing assumed interrelations among the ductless glands. Of course, the effect of activity of one gland on the activity of another would have to be accomplished with reasonable rapidity in order to be demonstrated by the galvanometer. It is quite possible that the influence of some glands on others, such, hypothetically, as that of the ovary on the thyroid, might be so gradual in its development as not to be recognizable by an electrical change. The conditions might be different in other relations in which quick effects are produced. Thus the adrenal glands are known to respond promptly to stimulation, and their secretion is rapidly influential. The thyroid glands also are capable of ready action, as shown by the brief latent period of their response after sympathetic stimulation. Consequently if the adrenals are capable of exciting the thyroid bodies, the effects should be demonstrable by means of the galvanometer.

Because in their experiments injection of adrenin increased the basal protein metabolism in dogs—a condition the same as that seen in hyperthyroidism and the opposite of that produced by removal of the thyroid glands—Eppinger, Falta and Rudinger (1) declared that the adrenal medulla probably exerts a stimulating influence on the thyroid gland. Since stimulation of sympathetic fibers induces in the thyroid gland a marked electrical change, as the paper before this one demonstrates, and since adrenin everywhere in the body duplicates the action of sympathetic impulses, the suggestion that adrenin and adrenal secretion may stimulate thyroid secretion seems highly reasonable. It is a suggestion easily put to test.

Figure 1 is the record made by the action current of the thyroid when 0.1 cc. of adrenalin (1: 100,000)[1] was injected (at *a*) intravenously. After a latent period (which in different instances varied from about 10 seconds upwards) the galvanometer was sent out to +77 and at the end of five minutes had slowly returned to +7, when the drum was stopped. This result and many others like it, have proved clearly that adrenin has a striking effect on the thyroid gland.

In a previous paper we have presented evidence that the nerve impulses delivered to the thyroid by sympathetic fibers do not act by lessening the blood supply, but stimulate the gland cells directly (2). This evidence is further supported by such results as are illustrated in figure 1. The amount of adrenalin injected in that instance was such as to produce a fall in blood pressure (3)—an effect due to relaxation rather than to contraction of the arterioles. We are justified, therefore, on the basis of this consideration in connection with considerations previously adduced, in drawing the conclusion that the thyroid action current evoked by small doses of adrenin is an indication of secretory activity.

Fig. 1. Thyroid action current resulting from the intravenous injection of adrenalin (0.1 cc., 1: 100,000) at *a*. Time recorded on base line here and in other figures in half-minutes.

This conclusion accords with recent observations by Osokin (4) that adrenin injections produce marked histological changes in the cells of the thyroid—changes which he regards as of secretory rather than of pathological character. It is not in agreement with Wiener's inference (5) that adrenin leads to a storage of thyroglobulin in the gland. But this inference was based on only two cases, and the figures from these cases are so different as to make hazardous any conclusion

[1] During the course of this investigation some observations by Mr. D. L. Rapport and one of us (W. B. C.) led to the discovery that stronger solutions of adrenalin (e.g., 1: 10,000) would affect other than gland tissue and smooth muscle. Thus if the galvanometer is connected with the muscles of the thigh, an intravenous injection of adrenalin (0.5 cc., 1: 10.000) will cause the galvanometer to move out continuously in the direction which would be determined by increase of the compensating current (cf. Cannon and Cattell: This Journal, 1916, xli, pp. 44, 45. This effect is not produced, however, by injections of small amounts (0.1 to 0.5 cc.) of adrenalin in dilutions of 1 to 100,000—amounts, however, which excite sympathetic endings.

whatever. The evidences from increase of protein metabolism after adrenin injection, from the increased histological changes of thyroid cells after such injection, and from the concordance of the action currents produced by sympathetic stimulation and by adrenin, all point clearly to a direct secretory influence of adrenin on the thyroid.

The question now arises, can the secretion of the adrenal glands call forth an action current from the thyroid? The well proved power of splanchnic impulses to stimulate secretion from the adrenals makes simple an experimental testing of this question. We bared the splanchnic trunks low in the thorax, tied them together tightly at the upper end of the bared portion, crushed the nerves central to the ligature, and slipped beneath them shielded electrodes. Also a bit of twine was looped around the inferior vena cava, close to its entrance into the thorax, so that by a slight pull the blood could be prevented from re-

Fig. 2. *A*, 3.34. Thyroid action current resulting from splanchnic stimulation, 35 seconds. *B*, 3.42. The same, except that return of blood through the inferior cava was prevented from the beginning of stimulation (30 seconds) until a minute later (at *x*). *C*, 3.55. Thyroid action current on releasing blood from abdominal vena cava after restraining it one minute.

turning through this vein to the heart. Now with the leading-off electrodes in place on the thyroid gland and on neighboring subcutaneous connective tissue, and the galvanometer at zero, the splanchnic fibers below the ligature were stimulated by means of a weak induced current. The record of such an experiment is shown in figure 2. The record in figure 2 *A* is that resulting when the splanchnic nerves were stimulated (at 3.34) with a weak tetanizing current for 35 seconds. The latent period was about 23 seconds from the beginning of stimulation. In figure 2 *B* (3.42) the nerves were stimulated just as they were in the case of *A*, but blood was prevented from returning from the abdomen through the inferior vena cava by a pull on the loop about the vessel. This tension was maintained for a full minute after the start of stimulation. The thyroid action current instead of appearing about 23 seconds after the stimulus was applied to the nerves (as in *A*),

did not appear until the loop about the inferior cava was released (at x)—more than a minute after excitation was commenced. In all probability the higher rise in B (to +45) compared with that in A (to +30) is due to the action of some substance accumulated during the damming back of the blood in the abdomen. That retention of blood in the veins of the abdomen does not by itself produce the effects seen after splanchnic stimulation is shown by figure 2 C. In this instance the loop about the inferior cava was pulled upon for a minute (3.55), and then the accumulated blood was released. During the next minute the galvanometer moved slowly out to +22, and then slowly back to zero. The rise is only half as high as it was in B, when the same procedure was followed, but without stimulation of the splanchnics. Furthermore the maximum effects were reached nearly thirty seconds later in C than in B.

A *B*

Fig. 3. *A*, 5.16. Thyroid action current following splanchnic stimulation, 30 seconds. *B*, 5.33. Absence of electrical effect on stimulating (for 60 seconds) the splanchnics after removal of the adrenal glands.

Splanchnic impulses may affect liver and pancreas as well as the adrenal glands. Discrimination of possible influences from these various sources is desirable. This can be done by noting the effect of splanchnic stimulation before and after removal of the adrenals. In figure 3 A is shown the record of a thyroid action current resulting from the stimulation of the splanchnic nerves (at 5.16). The galvanometer moved to +18 and to +29 and returned to +5. The adrenal glands were then carefully removed and thereupon the splanchnics were stimulated again (at 5.33) for one minute. The only effect was a wandering of the galvanometer from 0 to −4, back to 0, and to +4, as shown in figure 3 B. Subsequent injection of adrenalin in this instance induced a characteristic electrical change.

From the evidence illustrated above we are justified in concluding that the amount of adrenal secretion liberated by splanchnic stimulation is sufficient to excite the thyroid gland in a manner similar to its excitation by sympathetic impulses and by adrenin injections. Obviously the efficiency of sympathetic impulses in provoking activity of the thyroid might be greatly augmented by the simultaneous secretion of the adrenal glands. It is significant, therefore, that both the thyroids and the adrenals would be simultaneously stimulated by the diffusely distributed impulses of sympathetic neurons.

In an earlier paper (6) attention has been called to the emergency function of the adrenal glands in times of emotional stress, as distinguished from a routine function. The thyroid, likewise, doubtless has a routine function—one that serves to keep metabolism normal. That function can probably not be investigated by electrical methods. Because the gland is subject to sympathetic impulses, however, and because it responds to stimulation with great promptness, probably it also has an emergency function—one that is exercised particularly in times of emotional crisis. It may be that such an emergency function is an exaggerated form of the routine activity of the glands, but the determination of these suggestions must be left to future researches.

SUMMARY

Injection of small doses of adrenalin (0.1 to 0.2 cc., 1:100,000) evokes in the cat an action current of the thyroid gland.

Stimulation of the splanchnic nerves physiologically separated from the central nervous system likewise evokes an action current of the thyroid gland. This electrical change does not occur if the return of blood from the abdomen is prevented, but takes place promptly when the pent blood is released. The change also fails to appear after splanchnic stimulation if the adrenal glands have been previously removed.

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PANCREAS DEFICIENCY AND VASOMOTOR IRRITABILITY

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During the past three years the relation of various endocrine glands to the sympathetic nervous system has been under investigation in this Laboratory. That a relationship of this nature exists has been widely assumed. In view of the fact that the hormones of the body and the autonomic nervous system in general influence the same sort of functions—the so-called vegetative processes—the assumption is not at all improbable. On the other hand clean cut evidence of specific relationships between the endocrine system and either the sympathetics or parasympathetics is comparatively meagre. Particularly is this true in regard to the pancreas.

Nevertheless an important influence of the pancreas upon the sympathetic system has been asserted by the proponents of the "Wechselwirkung" theory of endocrine physiology. This assumption, in fact, forms one of the basic principles. Although the theory has largely collapsed of its own weight its occasional reappearance in supposedly authoritative clinical literature warrants some further attention to it (1).

The evidence most frequently cited of a relationship between the pancreas and the sympathetic system was recorded by Loewi in 1907 (2). After pancreatectomy both in cats and dogs it was observed that adrenin dropped into the conjunctival sac caused a dilatation of the pupil—a phenomenon that does not occur in the normal animal. The conclusion was drawn that the removal of the pancreas had caused augmented irritability of the sympathetic system, hence the pancreas normally has the function of depressing the sympathetics. Loewi further observed a similar mydriatic effect in 10 of 18 cases when adrenin was applied to the eyes of patients suffering from diabetes mellitus. In subsequent clinical literature this phenomenon is frequently referred to as the "Loewi reaction." The observation was soon confirmed by Biedl and Offer (3). Falta (4) observed adrenin mydriasis in 15 of

36 cases of diabetes. Other observers, however, have had less success. Cords (5) in 11 cases found only 3 positive. Bittorf (6) was able to obtain the reaction only twice in 10 cases and in each of these the patient was moribund. Pratt (7) found the reaction negative in three dogs with extreme atrophy of the pancreas and in a case of human diabetes. So far as we are aware observations of this sort constitute the only evidence in favor of Loewi's theory. The inconsistency of the phenomenon detracts from its significance, even granting the validity of the reasoning by which the theory was deduced.

That the deduction is valid, however, is open to question. The only condition in the sympathetic system that is known to cause an augmented adrenin reaction is peripheral *degeneration*. Elliott (8), Lichtwitz and Hirsch (9) and Biedl (10) have observed that when the sympathetic fibers to an organ are destroyed the responses to adrenin in that organ are materially increased. In view of this fact Loewi's observation as evidence is equivocal. One might equally well conclude that adrenin mydriasis, following pancreatectomy is due to depression of the sympathetic system or to a peripheral atrophy of sympathetic fibers. Indeed such an explanation has been offered to account for the glycosuria following pancreatectomy (11). On the whole it would seem to be an open question whether the pancreas has any specific relationship to the sympathetic nervous system.

In the hope of obtaining more satisfactory evidence on the point the experiments herein described were undertaken. As a typical component of the sympathetic the vasomotor system was selected as best adapted to quantitative study.

Technique. In all the experiments dogs were used as subjects. Ether anesthesia was employed throughout. By the technique described in previous papers of the series (12) the blood pressure and the reactions to standard doses of adrenin and nicotine were determined. These reactions afforded an index of the irritability of the peripheral and central and ganglionic portions of the vasomotor apparatus respectively (13). The drugs were administered intravenously. After the desired records were obtained the vessels were ligated and the incisions closed.

Then the pancreas was exposed through an incision, either in the median line or about 3 cm. to the right of this. The technique of pancreatectomy in the dog has often been described and needs no further discussion (14). The only points that seem worthy of emphasis are two: Minor hemorrhages should be ignored or stopped with

hot compresses; much time can be lost in needlessly ligating smaller vessels. Also the pancreatic ducts should be identified and ligated to prevent a possible escape of intestinal contents. The glands were removed completely except for an occasional small tag of tissue. A profuse glycosuria as well as emaciation in several of the dogs that were tested showed that a severe grade of pancreatic deficiency had been produced. The incisions were closed in three layers and protected with abdominal bandages. Comparatively little trouble with infection was experienced.

At different intervals from 3 to 22 days the blood pressure and adrenin and nicotine reactions were again recorded. In most instances two or three determinations after the initial one were made on each animal.

Results. The outcome of the experiments as a whole was somewhat difficult to interpret. The most striking feature was an unusual variability in the reactions to nicotine. In this type of investigation two precautions are essential to insure constancy of vasomotor response: the anesthesia must be held at a fairly uniform depth and the drugs must be injected at the same rate each time. But with the most careful attention to these points rather marked variability could not be avoided. The general trend of the series, however, is exemplified in the following protocol:

March 23, 1916. Dog anesthetized with ether. Cannulas inserted in right femoral artery and vein. Blood pressure and reactions to adrenin 1 cc., 2 cc. (1: 100,000) and nicotine 2 cc., 3 cc., 4 cc. (1: 4000) determined. Pancreas removed. Operation and recovery from anesthesia uneventful.

March 28. Cannulas inserted in left femoral artery and vein. Blood pressure and reactions to adrenin and nicotine taken as before.

March 29. Same procedures as March 28.

Results. (*Pressor reactions to drugs*)

BLOOD PRESSURE	BEFORE OPERATION	FIVE DAYS AFTER OPERATION	SIX DAYS AFTER OPERATION
	110	120	110
	mm. Hg.	*mm. Hg.*	*mm. Hg.*
Adrenin 1 cc.	32	38	36
Adrenin 2 cc.	42	62	50
Nicotin 2 cc.	8	23	10
Nicotin 3 cc.	30	33	22
Nicotin 4 cc.	62	34	26

In most cases the adrenin reaction was augmented—sometimes 50 per cent but occasionally it was unaffected or even slightly reduced. This variability of result corresponds with that reported when adrenin is applied to the conjunctiva except that augmentation of the pressor reaction is somewhat more frequent.

As to the nicotine reaction it is difficult to come to any conclusion as to magnitude on account of the variability encountered. When the proper precautions are taken a normal dog will give essentially the same response to a given small dose of nicotine several times in

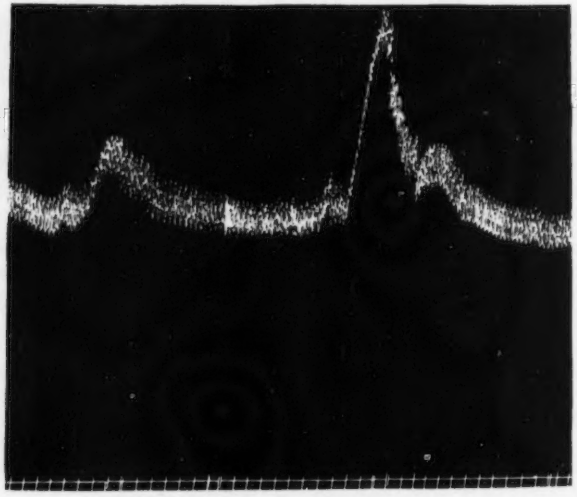

Fig. 1. Two successive vasomotor reactions to intravenous injections of nicotine 3 cc., 1: 4000. Interval, 5 minutes.

succession. After pancreas extirpation, however, no such constancy obtains. For instance note the reactions to two successive doses of nicotine shown in the accompanying figure. Our first interpretation of the phenomenon was that in the weakened condition of the animal its sympathetic motor neurons were especially susceptible to deleterious influences and were quickly depressed by the nicotine. With further experience, however, it was learned that the variability might go in either direction; sometimes the first and sometimes the second reaction was greater. As nearly as could be judged under these circumstances

the effect of the operation was on the whole to depress the nicotine reaction.

The blood pressure was either practically unaffected by the pancreatectomy or somewhat reduced. In only one instance was any augmentation noted. The pressure seemed to depend upon the general vitality of the animal rather than on any changes in irritability of the vasomotor apparatus.

Discussion. The outcome of the experiments is not without significance in its bearing on Loewi's theory. So far as pupillary diameter is concerned one has no way of judging what it would have been had the pancreas not been extirpated. The augmented reaction might conceivably in itself amount to over activity of the sympathetic system as Loewi and others have supposed (15). This might be true even though the sympathetic neurons were functioning merely to a normal extent because a given stimulus acting through a hyperexcitable effector would cause an augmented functioning of the given organ. As a matter of fact the proponents of the theory assumed that sympathetic functioning is normally due to stimulation by circulating adrenin—a theory no longer tenable (16). This is not, however, an assumption necessary to the theory if it be a fact that normal sympathetic impulses are transmitted through the medium of the same neurocellular receptive substance as that involved in reactions to adrenin. We are aware, however, of little specific evidence on this point. But in any case, our results indicate that the theory under discussion is untenable. There is no correlation between actual blood pressure and the augmented reaction to adrenin following pancreatectomy. The pressure is either unaffected or reduced. In no instance did we note any evidence of a true "sympathticotonie."

In connection with the observed instability of the reaction—due supposedly to variations in the irritability of the sympathetic motor neurons we are reminded of certain observations made by Carlson (17). This investigator found that waves of augmented irritability sweep over the nervous system during gastric hunger contractions. Luckhardt's (18) observation that hunger contractions after pancreatectomy are greatly augmented would seem to add some weight to the suggestion that both Carlson and we have encountered the same phenomenon. One of the most marked instances of variability in reaction was observed, however, in a dog which had considerable food in its stomach—when hunger was supposedly in abeyance.

Summary and conclusion. After pancreatectomy in the dog the

blood pressure remains either normal or somewhat depressed. The reactions to standard injections of adren n are usually augmented. The reactions to nicotine become markedly variable, at times augmented, but in general depressed. The observations do not support the theory that the pancreas normally exerts a depressing influence upon the sympathetic nervous system.

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VASOMOTOR REACTIONS FROM STIMULATION OF THE FLOOR OF THE FOURTH VENTRICLE

Studies in Vasomotor Reflex Arcs. III

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So far as we have been able to learn no serious attempt has been made to locate the vasomotor center since the work of Dittmar in 1873 (1). It is known that when the brain stem is gradually sliced away from above downward no effect on blood pressure is produced until the middle of the pons is reached, when the blood pressure begins to fall. When the sections pass through the upper part of the medulla the blood pressure falls to its lowest level, a level which is not affected by subsequent section. This experiment according to the generally accepted teaching, indicates the location of a vasoconstrictor center, which is responsible for the maintenance of blood pressure, in the lower part of the pons.

In recent years our knowledge of the vasomotor reflexes has been greatly extended by numerous researches which have dealt with the changes produced in the caliber of the blood vessels by the stimulation of various sensory nerves. In spite of all this work on the vasomotor reflexes little has been done to clear up our conception of the reflex arcs involved.

In connection with the more recent detailed studies of the vasomotor reflexes evidence has been presented to show that there is probably a vasodilator center more or less separate from the vasoconstrictor center (2). Porter (3) goes so far as to say that there is a vasotonic center which is distinct from the vasoconstrictor center. It would seem that there may be as many as three vasomotor centers and the question of their location and interrelation is the fundamental problem in this field of physiological research.

We wish to record very briefly by way of a preliminary communication, the results which we have obtained by direct stimulation of the

floor of the fourth ventricle. (4) These results suggest many interesting problems which must be investigated before they can be properly interpreted, yet the facts in themselves seem sufficiently interesting to merit a short report.

In six cats after ligation of the carotids the skull over the cerebellum was opened and the cerebellum removed, exposing the entire floor of the fourth ventricle from the aqueductus cerebi to the obex. The cats lost relatively little blood and showed no signs of shock, the blood pressure at the end of the operation being practically normal, varying from 100 to 160. Hemostasis was perfect and the floor of the ventricle was easily kept dry and clean. The stimulus was applied through a needle electrode. The second wire was attached to a piece of gauze moistened in normal salt solution and sewed under the skin of the cheek. The source of the current was a Stoelting inductorium No. 7090 through the primary of which a half ampere current was passed. This instrument has been roughly standardized by previous physiological experiments. With a half ampere current passing through the primary and with the secondary coil at 15 it delivers through standard platinum electrodes a stimulus just sufficient to produce a typical depressor reflex from the sciatic nerve. The coil must be moved up to 5 to produce from the sciatics the maximum pressor reflex. In these experiments we worked with the secondary coil at positions varying from 15 to 10, that is to say with weak stimuli which if applied to the sciatic through standard platinum electrodes would produce a drop in blood pressure.

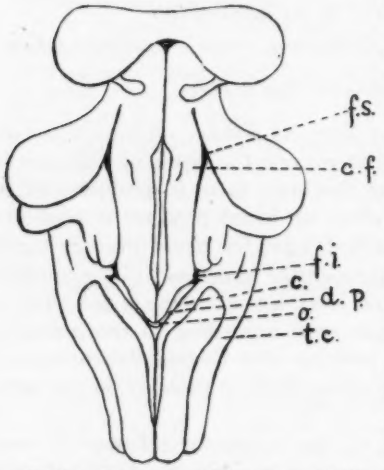

Fig. 1. Diagram of the floor of the fourth ventricle of the cat. *f.s.*, fovea superior; *c.f.*, colliculus facialis; *f.i.*, fovea inferior; *c.*, clava; *d.p.*, depressor point; *o.*, obex; *t.c.*, tuberculum cinereum.

On going over the floor of the fourth ventricle point by point with the needle electrode we found that there were two points which responded to this stimulation by producing marked changes in blood pres-

sure. The first of these is the apex of the ala cinera or the fovea inferior, figure 1. When the electrode is applied to this point a marked rise in blood pressure occurs, often as much as 40 mm. Hg., figure 2. This response may or may not be accompanied by respiratory inhibition, excessive respiration, cardioinhibition or slight movements of the head according to the stage of anaesthesia and other variable factors. The rise of blood pressure is characteristic and specific. This pressor point, as we shall call it until we know more about its real nature, does not seem to be more than a millimeter in diameter but is readily located because the ala cinerea and fovea inferior are both readily distinguished.

The second spot, which we shall call the depressor point, is located in the area postrema just lateral to the obex. The needle is inserted under the clava just lateral to the obex, figure 1 d.p. There is no sharply defined anatomical demarcation of this as was the case with the pressor point and some experience is required before one can locate it with regularity and ease. It also seems to be relatively small and sharply localized. Stimulation here causes a marked drop in blood pressure often amounting to 35 mm. Hg., figure 2.

We have sometimes obtained a rise in blood pressure from stimulation in the region of the facial colliculus, i.e., in the neighborhood of Ditmar's vasoconstrictor center, and we have also obtained a good pressor curve from stimulation of the tractus spinalis of the trigeminal nerve, i.e., the tuberculum cinereum on the dorsolateral aspect of the medulla.

The most characteristic and consistent reactions are obtained from the pressor and depressor points. These are located not more than 3 mm. apart and the same strength of stimulus, which applied to the one sends the blood pressure up 30 to 40 mm., when applied to the other sends the blood pressure down to 25 to 30 mm. These reactions closely simulate true pressor and depressor reactions.

Aside from these two points and the region of the facial colliculus, which gives somewhat inconstant pressor reactions, we could find no part of the floor of the fourth ventricle which would give vasomotor reactions with the weak stimuli used throughout these experiments. Stronger stimuli were not used as it was felt that results obtained with them would not be reliable.

The same results were obtained with the vagi cut as with them intact, and under curare and ether as with ether alone, except that curare tends to decrease the drop obtained from the depressor point. In one cat under curare, however, a marked drop was obtained. It will be

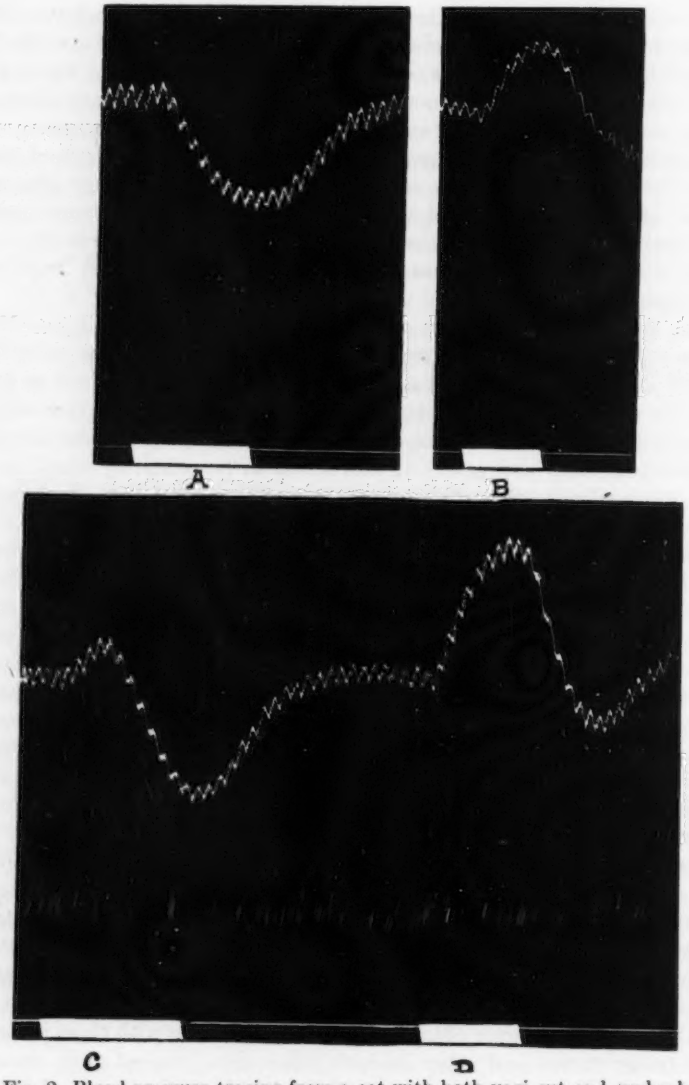

Fig. 2. Blood pressure tracing from a cat with both vagi cut and under both ether and curare: *a*, stimulation of the depressor point secondary coil at 11; *b*, stimulation of the pressor point secondary coil at 11; *c* stimulation of the depressor point secondary coil at 10, slight preliminary rise caused by escape of current to pressor point; *d*, stimulation of pressor point secondary coil at 10.

remembered that curare has the effect of diminishing or obliterating the depressor reactions obtained from stimulating afferent nerves except in the case of the depressor nerve. The effect of the drug on the depressor point is therefore in line with what is known of its action in general.

All six cats gave the same results: rise of blood pressure from the pressor point, and fall in blood pressure from the depressor point. The rise and fall were always of considerable magnitude.

It would seem that there are two possibilities in regard to the nature of the points which have been found to yield vasomotor reactions on stimulation. It is quite possible that they may represent the central connections or receptive nuclei of afferent vasomotor fibers. In that case they represent a part of the afferent limb of the corresponding reflex arc. This is almost certainly the interpretation to be placed on the reactions obtained from the tractus spinalis trigemini, which is the direct upward continuation of the tract of Lissauer, which serves as the afferent path for pressor impulses in the spinal cord (5).

It is quite possible that the same interpretation applies to the pressor and depressor points which have just been described. The pressor point may represent the termination of the afferent pressor fibers of the vagus and the depressor point the termination of the afferent depressor fibers of the vagus, since both of these points are closely related to the known nuclei of the vagus nerve. The responses which were sometimes obtained from the region of the facial colliculus may have come from a stimulation of the main sensory nucleus of the trigeminal nerve which is not far distant.

On the other hand it seems possible that the depressor point may be a true vasodilator center, the pressor point a true vasoconstrictor center. In this case it would seem probable that there was also a vasotonic center in the region of the facial colliculus which was also capable of responding to direct stimulation but in a less characteristic manner than does the pressor point.

It is clear that the electrical stimulation of the floor of the fourth ventricle offers a rich field for the investigation of vasomotor reactions. Irrespective of the interpretation, which in the light of future researches is finally placed on the pressor and depressor points, a careful analysis of the reactions obtained from stimulating these points will throw considerable light on the whole question of the vasomotor reflexes.

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THE NATURE OF THE SPLANCHNIC RISE IN BLOODPRESSURE

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In 1897 Biedl (1) observed that the stimulation of the splanchnic nerve produces a vasodilatation in the suprarenal bodies, and, although unable at that time to detect vasoconstrictor fibers, the existence of fibers of this kind was inferred from the changes which result if adrenalin is injected directly into this organ. Moreover, a quantitative determination of the adrenal secretion made it appear highly probable that its formation and discharge into the venous current, is subject to the control of the nervous system, the splanchnic nerves being the secretory and vasomotor pathways of these glands. A year later Dreyer (2) succeeded in proving the existence of the vasoconstrictor mechanism in a more convincing manner and also showed that the stimulation of the splanchnic fibers leads to a liberation of adrenal substance which is quite independent of the alterations in the vascularity of these glands.

Since the publication of these papers the existence of secretory nerves to the adrenals has been established with much greater definiteness by Ascher (3), Cannon (4), Elliott (5), and Von Anrep (6). The experiments of Ascher were made upon rabbits whose abdominal organs, with the exception of the liver and suprarenal bodies, had been removed. It was noted that the stimulation of the splanchnic nerve resulted in a rise in bloodpressure only so long as the suprarenal bloodvessels were left intact. The hypothesis that the adrenal glands share in the production of the phenomena associated with emotional states, was tested by Cannon and de la Paz by immersing strips of intestinal muscle in Ringer's solution to which was added the defibrinated blood from cats that had been kept either in enforced quietude or had previously been excited. The blood from the quiet animals always failed to relax the muscle, while the blood gathered after emo-

tional disturbances, produced a typical relaxation. In order to prove that the cause of this difference is dependent upon a discharge of adrenin, experiments were made upon the relative relaxing power of blood from different systemic veins and that of the suprarenal veins. The former type of blood proved ineffective, moreover, the relaxation failed to appear, if the blood had been obtained from "excited" animals whose adrenal bodies had previously been removed.

It has been observed by Johansson (7) that the rise in the arterial bloodpressure accompanying the stimulation of the splanchnic nerve, does not consist of a simple rise and fall, but shows two elevations. Lehndorff (8) states that the first of these is caused by a vasoconstriction of the bloodvessels in the organs innervated by the aforesaid nerve, and that the second summit is produced partly by an acceleration and augmentation of the heartbeat, and partly by a constriction of certain peripheral vessels. Elliott who investigated this question in the cat, ascribes the second phase of the rise to a discharge of adrenal secretion into the circulation, because the excitation of the splanchnic nerve failed to produce this elevation after the extirpation of the suprarenal glands. These results have been fully confirmed by Von Anrep, and furthermore, this author was able to show that the general vasoconstriction upon which the second rise depends, also occurs in certain organs and parts after their denervation. This fact clearly establishes the function of adrenin as an aid to the direct or nervous agency of vasoconstriction, and also explains the peculiar local reaction of the bloodvessels to internal pressure first noted by Bayliss (9).

The present experiments have been undertaken for the purpose of determining in how large a measure the splanchnic bloodvessels contribute to the first and second phases of the rise in the systemic bloodpressure following the stimulation of the splanchnic nerves. The dogs used in these tests were anesthetized with ether. The abdominal cavity having been opened in the median line, the diaphragm was pierced in the region of the crura. Artificial respiration having been instituted, the thoracic sympathetic nerve of each side was isolated and placed in shielded electrodes directly above its point of division into the greater splanchnic and abdominal sympathetic nerves. In order to avoid traction upon the nerve during the succeeding manipulations, the electrodes were introduced through the lateral extent of the lowest intercostal space. The current was applied to the intact nerve in some instances, and in others, only to the distal end of the divided nerve. The stimulations were of moderate strength and duration.

The bloodpressure was recorded by a mercury manometer connected with the femoral artery. A second manometer was added at times to register the pressure in the carotid artery. The following bloodvessels were placed in loose ligatures: the right and left renal arteries and veins, the mesenteric arteries, the coeliac artery, the portal vein at the hilus of the liver, and the right and left suprarenal veins.

By measuring the bloodflow through different abdominal organs during stimulation of the thoracic sympathetic, Burton-Opitz (10) has shown that the right nerve innervates, the right kidney and suprarenal capsule, as well as the stomach, intestine, spleen and pancreas, while the left nerve governs the left kidney and suprarenal body, and in addition, the portal organs just enumerated. In accordance with this distribution of the splanchnic fibers, it was thought certain that the exclusion of all of these organs from the circulation, together with the division of the hepatic plexus, would completely nullify the rise in bloodpressure which accompanies the stimulation of these nerves. Moreover, by permitting only the suprarenal veins to remain open, we hoped to obtain a means for differentiating between the effects of the discharged adrenin upon the bloodvessels of the splanchnic organs and upon those situated in other parts of the body.

At the outset, the right and left nerves were tested as to their relative effectiveness; currents of the same strength and duration being applied to them alternately. We have obtained more characteristic and more decided rises by the excitation of the right nerve, and naturally, by far the greatest amplitude of reaction was noted when both nerves were stimulated simultaneously. In a comparison of this kind, however, it must be taken into account that the rises obtained early in the course of an experiment, are generally much larger that those gotten later on, unless the intervals are sufficiently long to permit of not only a complete relaxation of the bloodvessels, but also of a thorough restoration of the secretory power of the adrenal glands.

In accordance with the preceding statements it cannot surprise that the stimulation of the splanchnic nerves during the occlusion of the suprarenal veins failed in all cases to elicit the secondary rise in bloodpressure. Quite similarly, it is possible to delay the appearance of the second phase by temporarily blocking the suprarenal vein of the gland stimulated. As is clearly indicated in figure 1, an obstruction for only a few seconds made directly at the beginning of the stimulation, suffices to retard the development of the second elevation to such an extent, that it occurs much later in the course of the vascular relaxa-

tion. In this way the first and second phases may be separated more widely so that a distinct notch or step appears between them. The general outline of the curve may thus be changed almost at will.

Strehl and Weiss (11) have stated that the ligation of the suprarenal bloodvessels is followed by an immediate fall of pressure, while the release of the ligature is succeeded by a rise. This result would tend to show that the adrenal secretion is poured out constantly to serve as a stimulant to the sympathetic nervous system and to the smooth

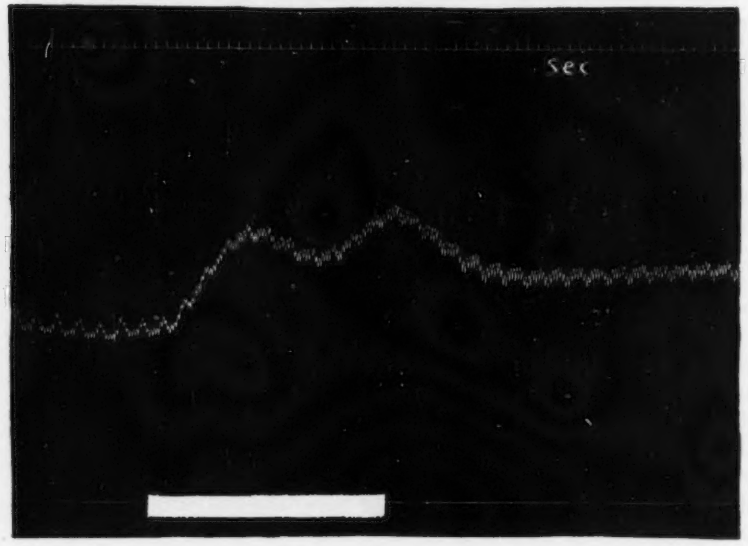

Fig. 1. Stimulation of both thoracic sympathetic nerves. Separation of the phases by brief occlusion of the suprarenal veins.

musculature of the bloodvessels. Young and Lehman (12), on the other hand, have observed but a very slight fall in pressure, while Hoskins and McClure (13) report that the ligation of the adrenal glands causes no immediate fall in bloodpressure. The experiments of these authors, however, were not continued for a longer period than ten or thirty minutes and a subsequent removal of the ligatures was regarded as useless, because it was thought likely that the checking of the adrenal circulation results either in a depression of the function of the glands, or in an accumulation of elaborated secretion. These tests, there-

fore, do not favor the view that the constrictor and other sympathetic fibers are constantly kept under the tonic influence of the adrenal bodies.

In the course of these experiments we have had occasion to test the effect of obstructing the suprarenal bloodflow a number of times. In each case we have done so by tightening the loose ligatures which had previously been placed around the vein centrally as well as distally to the gland. Under ordinary conditions, however, it suffices to ligate only the central portion of the vein, because it seems that an escape of adrenin through distal anastomosing bloodvessels cannot readily be effected. At least, if a reversal of the venous flow of this region follows the blocking of the suprarenal vein near its outlet, the adrenal secretion reaches central channels in a wholly ineffective condition, as is evidenced by the absence of the second phase of the rise in the general bloodpressure. In testing this point we have employed solely currents of very moderate strength and duration, and hence, these results should not be applied to those cases in which the accumulated quantity of adrenal secretion has been greatly augmented by repeated stimulations of the splanchnic nerve.

While we cannot substantiate the observation that the ligation of the suprarenal bloodvessels is followed by an immediate and marked fall in pressure, the largest number of our records unmistakably indicate a downward tendency on the part of the pressure. The most pronounced drop that we have obtained is represented in the curve illustrated by figure 2. It can readily be seen, however, that its value is inconsiderable and that it develops with extreme slowness. For this reason it is easily modified and destroyed by changes in any one of the factors upon which bloodpressure is dependent; and hence, it is usually compensated for within a comparatively short time. Thus, it may happen that the initial decline is soon followed by a pressure which even surpasses that registered before the ligation.

The fact that the subsequent release of the adrenal secretion produces a rise in pressure is considered of little significance by Hoskins and McClure, because it is regarded as probable that the checking of the secretion results either in a depression of the glands, or in an accumulation of elaborated adrenin. In either case, it is stated, the removal of the ligatures does not restore normal conditions. While these possibilities must be taken into account, it seems, however, that indirect evidence of this kind should not be disregarded altogether. The same objection might indeed be raised against very many physiological methods and results.

It has been our experience that, if the ligation of the suprarenal veins was continued for only a brief period of time without stimulation of the splanchnic nerves, the release of the ligatures was not followed by a marked elevation of the bloodpressure. A certain gradual upward tendency of the pressure was, however, clearly recognizable, but again, the rise as a whole did not assume significant proportions. If the obstructions were continued for fifteen to thirty minutes, much more immediate effects were obtained which consisted in brief but quite distinct elevations of the pressure, but naturally, the question whether the adrenin has accumulated in these cases in consequence

Fig. 2. Ligation of right (*A*) and left (*B*) suprarenal veins.

of constant or occasional stimuli, does not find an answer in these data. Reactions of very much greater amplitude resulted, if stimulation of the splanchnic nerves had been resorted to during the obstruction. As is apparent in figure 3, on releasing the elaborated secretion first from one and then from the other gland (at *A* and *B*) two successive elevations in the pressure resulted, the amplitude of which preserved a direct relationship to the strength and duration of the preceding stimulations. The latent periods are well marked in both cases.

In accordance with these results it seemed possible to alter the general outline of the splanchnic rise in bloodpressure still further by

Fig. 3. Removal of the ligatures from right (*A*) and left (*B*) suprarenal veins.

excluding the effects of the primary vasoconstriction. This end we have sought to attain by temporarily ligating all of the arteries supplying the organs innervated by the splanchnic nerves, namely, the right and left renal, the superior and inferior mesenteric, and the coeliac. In addition to these bloodvessels, ligatures were also applied to the renal and portal veins, and, in a few cases, also to the hepatic vein, because it was thought possible that the resulting vasoconstriction might force a part of the residual blood of these organs into central channels; thereby tending to preserve the first rise.

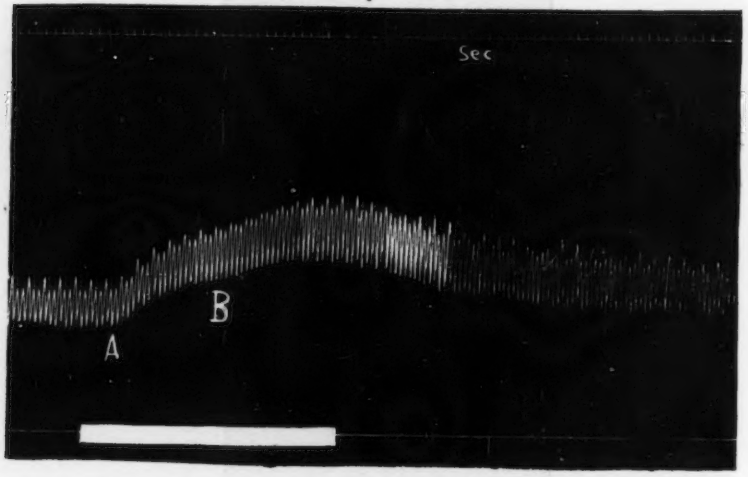

Fig. 4. Stimulation of the distal ends of the thoracic sympathetic nerves (14 cm.). All blood vessels open.

The successive exclusion of these organs from the circulation increased the general bloodpressure by about 35 to 45 mm. Hg, the pressure retaining its high value without much compensation. The greatest rise resulted after the occlusion of the mesenteric arteries. At best it amounted to about 20 mm. Hg, while that produced by the ligation of the coeliac axis, amounted to about 15 mm. Hg. By removing the ligatures one at a time quite similar step-like decreases in pressure were obtained which showed the same relationships toward one another. In all these cases, however, the portal vein must be closed last, otherwise a large part of the blood will be stagnated in

the tributaries of this vessel with the result that the general bloodpressure assumes a level much below normal.

The splanchnic organs having been excluded from the circulation in the manner just described, the stimulation of only one or both thoracic sympathetic nerves produced in most of the animals a very marked reduction in the amplitude of the first elevation. In a few cases the first rise disappeared altogether. The second elevation, on the other hand, was never absent, although always smaller in size. This change, however, is explicable without difficulty upon the basis that the second summit is really founded upon the first and hence, its conspicuousness must decrease in accordance with the loss in the amplitude of the first. The accompanying figures 4 and 5 are intended to illustrate this point more fully. The former was recorded with the

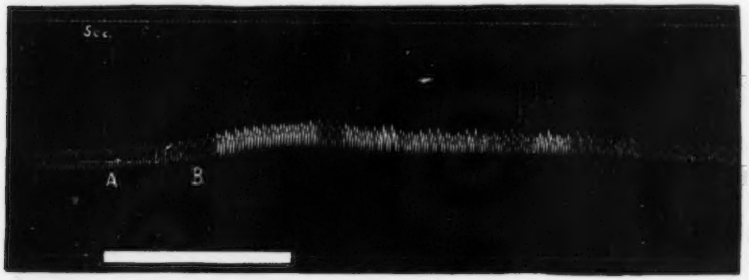

Fig. 5. Stimulation of the distal ends of the thoracic sympathetic nerves (14 cm.). Only suprarenal veins are open.

bloodvessels opened and the latter after they had been temporarily occluded. In each case both nerves were stimulated simultaneously with a current of medium strength and duration. The second rise which, as has been stated previously, is dependent upon the discharge of adrenin, begins about fifteen seconds after the onset of the stimulation at *B* and ends sometime after the cessation of the excitation. The first rise which is caused by the primary vasoconstriction of the bloodvessels of the "splanchnic" organs, becomes evident at *A* within two or three seconds after the beginning of the stimulation. Figure 6 is intended to illustrate the results which were obtained in only two of the eight animals experimented upon. No rise whatever is discernible in this case until the adrenin has had sufficient time to produce the characteristic second elevation (at *B*).

The reduction in the amplitude of the rise in bloodpressure is quite apparent in all cases, but what concerns us most at this time is the fact that the primary elevation cannot always be completely destroyed by the ligation of the splanchnic bloodvessels. In fact, other tests have given even greater rises, equalling 8 to 12 mm. Hg. Clearly, therefore, the most important part in the production of the first rise is played by the bloodvessels of those organs which are innervated by the greater splanchnic nerves. Obviously, however, the excitation of the thoracic sympathetic nerve must also affect another vascular

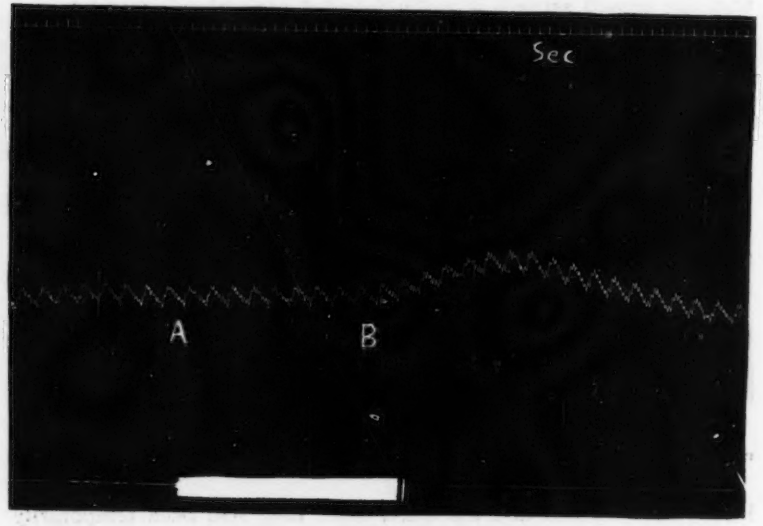

Fig. 6. Stimulation of the distal ends of the thoracic sympathetic nerves (14 cm.). Only suprarenal veins open.

area which is not situated in the domain of the splanchnics. This fact was brought out more clearly by ligating the suprarenal veins in addition to the bloodvessels mentioned previously. The stimulation of the thoracic sympathetic nerves produced under these conditions no elevation whatever in two animals, but a very evident first rise in six animals.

As is well known, the thoracic sympathetic nerve divides close to the diaphragm into the greater splanchnic and abdominal sympathetic nerves. In its course downward, the latter gives off branches which

enter the ramification of nerves in the vicinity of the suprarenal capsule. It is true, however, that the size and location of these nerves is subject to variations; in fact, in some animals the abdominal sympathetic is very rudimentary.

In order to trace these extra-splanchnic vasomotors, the existence of which seems to have been definitely established by the preceding experiments, we resorted first of all to division of both greater splanchnic nerves. All the different splanchnic bloodvessels having been ligated, the stimulation of the thoracic sympathetic nerves now led to results

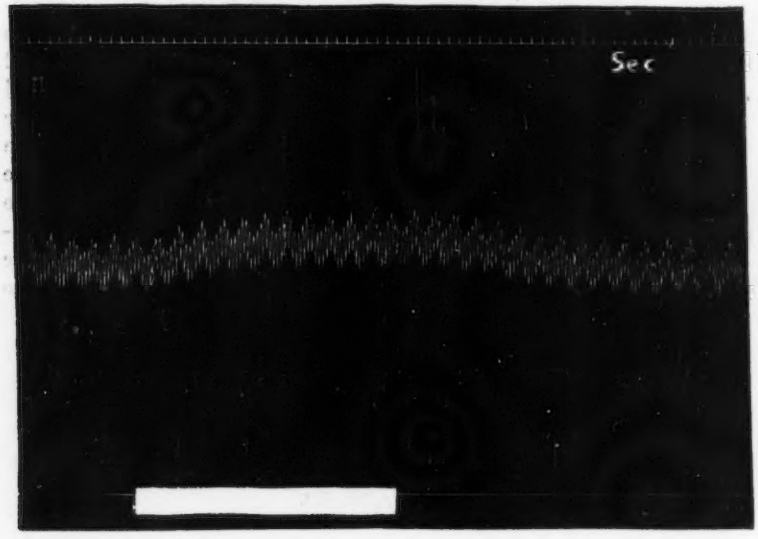

Fig. 7. Stimulation of the thoracic sympathetic nerves (12 cm.). Greater and minor splanchnic nerves are divided.

which were in no way different from the preceding. In the largest number of instances a clearly perceptible first rise developed, while in those animals in which no reaction was obtained, the abdominal nerve was poorly developed.

This procedure, however, does not exclude the possibility that impulses generated upon the thoracic sympathetic reach the splanchnic organs by way of the lesser splanchnic nerves, although the ligation of the bloodvessels would prevent an effect of this kind from making itself felt centrally. In order to avoid this difficulty, the stimulations

of the thoracic sympathetic nerve were also repeated after division of the greater and lesser splanchnic nerves, and after all the bloodvessels had been ligated. As is shown in figure 7, the first phase of the rise in bloodpressure persisted in most of these tests, a low and long elevation now taking the place of the formerly very conspicuous double rise.

These results indicate that the abdominal sympathetic nerve embraces vasoconstrictor fibers which govern the vascularity of parts not situated within the realm of the greater and minor splanchnic nerves. Hence, the first elevation of the rise in the general bloodpressure resulting from stimulation of the thoracic sympathetic nerve, is in part dependent upon the constriction of the bloodvessels innervated by this nerve. It is true, however, that the power of the abdominal branch of the sympathetic is very much less than that of its splanchnic division, a difference which no doubt is dependent upon the relative size of the vascular areas controlled by these nerves. While we have not been able so far to determine the seat of this reaction, we have thought it probable that the abdominal sympathetic may be functionally connected with the lumbar and sacral networks of the sympathetic system and may thus serve as a constrictor of the bloodvessels of the lumbar muscles.

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DEPRESSOR ACTION OF THE THORACIC SYMPATHETIC NERVE AND ITS BRANCHES

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It has been noted repeatedly that the rise in the systemic bloodpressure occasioned by stimulation of the splanchnic nerve, is followed at times by a fall below normal which may in turn be succeeded by a slight rise. This phenomenon has generally been regarded as being dependent upon a compensatory adjustment in distant parts of the vascular system. It is true, however, that the fall attains at times a conspicuousness which actually suggests a reflex dilatation. A reaction of this kind is represented in figure 1. The current was applied in this case to the intact thoracic sympathetic nerve, directly above its point of division. The stimulation was followed at first by a rise in bloodpressure consisting of two phases (at *A* and *B*), while subsequent to the second elevation the pressure dropped rather abruptly (at *C*), giving rise to an effect which closely resembles a true depressor action.

In other parts of the body Dastre and Morat (1) have observed that the excitation of a so-called constrictor nerve produces a constriction of the bloodvessels innervated by this nerve and that this reaction is followed by a dilatation. This after-affect to which the name of "surdilatation" has been given, is considered by them to be an exhaustion phenomenon. It is assumed that the excitation increases the tonicity of the muscle fibers of the vessel wall in such a degree that they become fatigued and temporarily relax.

Bradford (2) has shown that the vasomotor nerves of the kidney leave the spinal cord through the anterior roots from the fourth to the thirteenth dorsal vertebrae. These fibers embrace constrictors and dilators, but the former are the best developed by far so that it is difficult to obtain clear evidence of vasodilation by stimulation of a spinal nerve. Excitation of the central end of an intercostal nerve was fol-

lowed by a contraction of the kidney, the same result being usually obtained by reflex stimulation of the spinal nerves. As far as the splanchnic nerve is concerned, the statement is made that this nerve embraces constrictor and dilator fibers for the abdominal bloodvessels, because it was possible to induce a fall in the general pressure which was frequently succeeded by a rise. In accordance with the mode of preparation, these effects have been attributed by Bradford to a

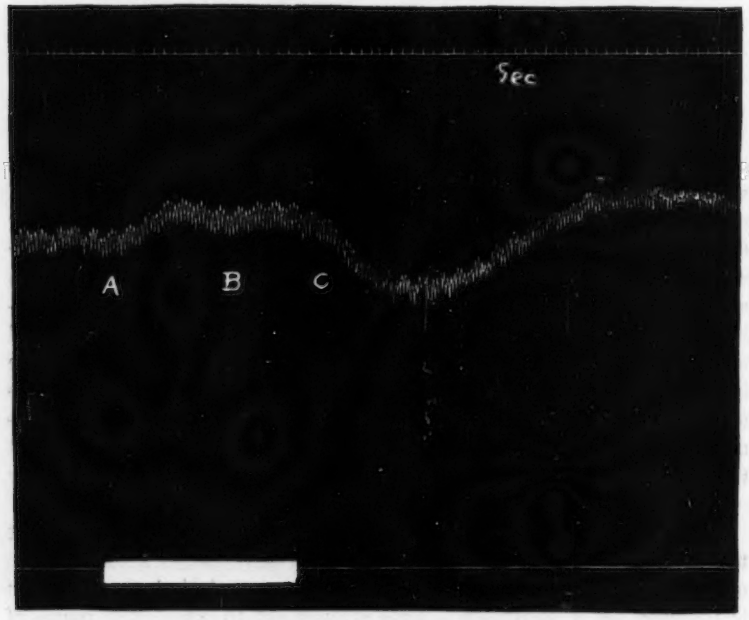

Fig. 1. Stimulation of the thoracic sympathetic nerve (14 cm.)

peripheral and not to a central excitation. The shrinking of the kidney, for example, is explained by assuming that the stimulation of the splanchnic nerve acts mainly upon the bloodvessels of the intestine, so that the dilatation in the latter organ in turn produces a diminution in the bulk of the kidney. This reaction is said to take place even if the renal vessels take no part in the dilatation, because a very large quantity of blood is diverted into the intestinal channels. Thus, it is believed that these dilator phenomena are dependent upon

a direct and active dilatation of the intestinal bloodvessels which in turn affects the kidney in a passive manner, although it is not denied by Bradford that this organ may also show some active dilatation.

The phlethysmographic experiments of Hallion and Francois-Frank (3) made upon loops of intestine, have shown that the vasoconstrictor fibers of the intestinal bloodvessel arise below the fifth dorsal nerve, while the dilators take their origin in the eleventh, twelfth and thirteenth dorsal communicating rami as well as in the first and second lumbar nerves. The stimulation of the distal ends of these fibers produces an increase in the volume of the intestine which may and may not be initiated by a slight decrease. The former reaction is associated with a rise and the latter with a variable fall in the aortic pressure.

I have cited the work of these authors somewhat in detail in order to show that it is wholly concerned with direct peripheral effects, namely with the vasoconstrictions and vasodilatations occurring in the kidneys and intestines. In the succeeding pages I shall endeavor to prove that the "splanchnic" nerve also serves as an afferent path and that its excitation leads to a general vasodilatation. For this reason, this nerve may be regarded as the depressor for the abdominal organs.

Opposite to the thirteenth rib, the thoracic sympathetic divides into the greater splanchnic and abdominal sympathetic nerves. Distally to this point, the border-sympathetic most generally consists of only a small number of fibers which eventually connect with the lumbar ganglia, but at times it retains a size which compares favorably with that of the splanchnicus.

The dogs made use of in these experiments were anesthetized with ether. Having opened the abdominal cavity, the diaphragm was pierced on both sides in the regions of the crura. The bloodpressure was registered by a mercury manometer which was connected with the left femoral artery. The stimulation of the nerves was effected with a Du Bois-Raymond coil and ordinary shielded electrodes; a three-volt battery furnished the power.

To begin with, the electrodes were applied to the intact thoracic sympathetic nerve about 1 cm. above its division into the greater splanchnic and abdominal sympathetic nerves. The coils were at first widely separated so as to be beyond the threshold value of the current. They were then brought closer together after each stimulation, until eventually the first indications of a fall in bloodpressure appeared. If this end could not be attained with weak currents, the frequency of vibration of Neef's hammer was reduced gradually

by increasing its contact distance. A graduated inductorium and Schlagwähler were never required in these tests to bring about the result just mentioned.

The decreases in the pressure could be rendered more conspicuous by increasing the strength of the current somewhat beyond its threshold value. When, however, a certain maximum had been reached, the fall was invariably superseded by a rise in the pressure which exhibited the characteristic double elevation. In all these tests it was necessary to separate the individual stimulations by intervals of a few minutes, otherwise the succeeding falls in pressure lost much of their original conspicuousness. This difficulty which clearly suggests that the dilators are much more susceptible to fatigue than the constrictors, can be overcome by using a somewhat stronger current for the succeeding stimulation. For example, if a marked fall in pressure has been obtained with a distance of coils of 25 cm., an increase, say, to 20 cm. will tend to preserve the amplitude of the drop without necessitating a long period of rest. Quite similarly, it was possible to produce a series of rises and falls in rather rapid succession by simply increasing or decreasing the distance of the coils.

While the amplitude of the fall in pressure could be varied by changing the strength of the current, it was rather difficult to drive the pressure beyond a certain low level. In the tests here at hand, the decreases frequently amounted to 20 mm. Hg., but rarely to more than 30 mm. Hg. The maximal values obtained equalled 30 to 40 per cent of the normal pressure. In general, therefore, it may be said that these reactions bear every resemblance to the fall in pressure which is occasioned by the excitation of the depressor cordis (fig. 2). In no case, however, could a marked decrease in the frequency of the heart be made out, although a tendency of this kind seemed always to exist. A fair average result is seventy normal beats to sixty-five beats during the period of depression.

These effects were obtained with even greater certainty if both thoracic sympathetic nerves were stimulated simultaneously, and were also gotten if the intact greater splanchnic or the intact abdominal sympathetic nerve was subjected to the current. While the general character of the reaction strongly suggested in all these cases a reflex vaso-dilatation; account has also been taken of the possibility that the adrenal glands may have been concerned in the production of this phenomenon.

It has commonly been held that adrenalin possesses solely vaso-

constrictor properties (4); in recent years, however, many new facts have been ascertained which tend to prove that in very minute doses this agent also manifests the power of lowering the pressure by relaxing the bloodvessels. Evidence of this kind has been obtained by Bardier and Fränkels (5), Moore and Purington (6), Meltzer (7), and Pari (8); in fact, Cannon and Lyman (9) have succeeded in pro-

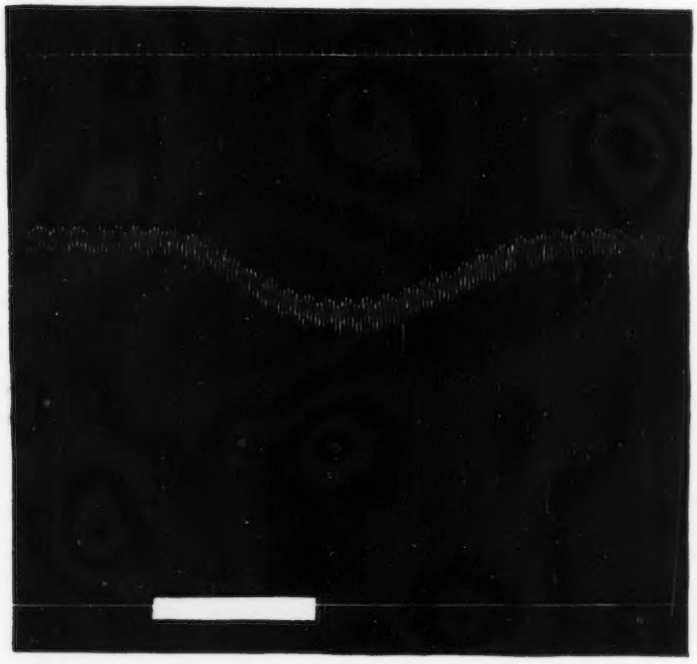

Fig. 2. Stimulation of the intact thoracic sympathetic nerve (25 cm.)

ducing a fall in bloodpressure in cats after exclusion of the splanchnic organs by simply stimulating the splanchnic nerve or the gland itself. Reasons are given by these authors for assuming that this dilator effect is not of central origin, is not dependent upon a blocking of the constrictor impulses and is not caused by the stimulation of supposed vasodilator sympathetic endings. Thus, adrenalin is said to possess opposite actions in accordance with the state of the smooth muscle

fibers of the bloodvessels. If these elements are tonically shortened this agent relaxes them, while if relaxed, it contracts them.

It has been observed previously (10) that the latent period intervening between the stimulation of the greater splanchnic nerve and the beginning of the second phase of the rise in the general bloodpressure amounts to about 13 seconds. It may be assumed, therefore,

Fig. 3. Mechanical stimulation of the central end of the thoracic sympathetic nerve.

with reasonable certainty that a dilator effect which is caused by a liberation of adrenal secretion, must require an equally long time to make itself felt. An inspection of the records, however, shows that the latent periods seldom exceed a length of 3 to 4 seconds, in spite of the fact that they tend to be somewhat longer than those initiating the splanchnic rise in bloodpressure.

While probably no additional evidence is required to show that this

depressor action is not dependent upon a liberation of minimal quantities of adrenin, I ligated in one animal the suprarenal veins centrally and distally to the glands and removed practically all of their tissue. No change whatever in the character of the fall in bloodpressure was noted.

Having in this way excluded the adrenal bodies as causative factors in the production of this vasomotor reaction, I proceeded to establish

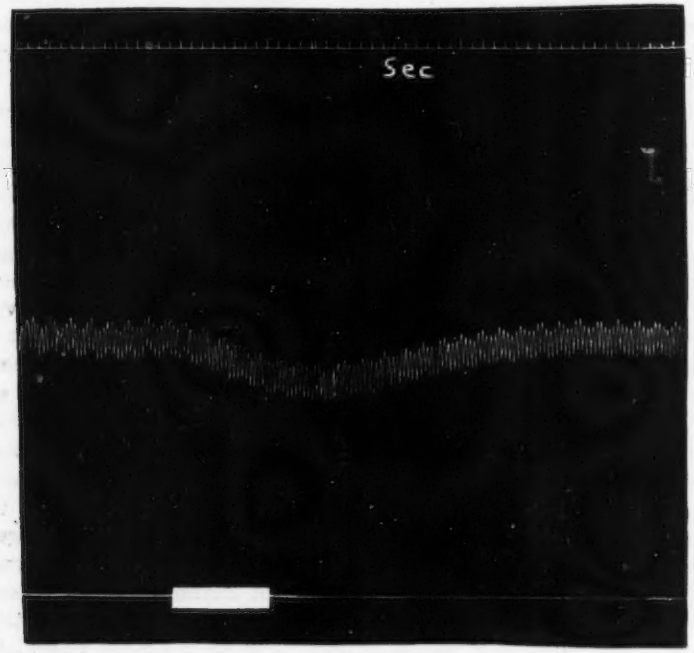

Fig. 4. Stimulation of the central end of the left thoracic sympathetic nerve, the opposite nerve and both vagi nerves having been divided (22 cm.).

its reflex character in a more convincing manner. To begin with I divided the greater splanchnic nerve of the left side in close proximity to the suprarenal plexus and stimulated its central end in the manner described previously. Very pronounced falls in bloodpressure were obtained. I then divided the abdominal sympathetic nerve above the first minor splanchnic nerve, and repeated the stimulations, using

the central end of this nerve. While the reactions now showed a reduction in amplitude, their general character remained the same. And finally, I divided the thoracic sympathetic nerve at a point about 1 cm. above its bifurcation and applied the electrodes to its central end. The stimulations invariably led to very characteristic falls in pressure. Precisely the same results were obtained subsequently with the right thoracic sympathetic nerve and its branches, and hence, the conclusion may justly be made that the aforesaid nerve serves as the depressor nerve for the abdominal viscera. It might also be mentioned that a mechanical stimulation of the central end of this nerve sufficed in many cases to bring this result about (fig. 3).

In accordance with the large amplitude of this depressor reaction, it was thought probable that extensive areas of the vascular system are involved, but naturally, one might feel inclined to attribute the principal effect to the bloodvessels of the abdominal organs. Without desiring to establish accurate quantitative differences, as could probably be done with the help of a calibrated inductorium (11), I have sought to determine the seat of this reaction in a perfectly general way as follows: Having divided both thoracic sympathetic nerves, the electrodes were applied to the central end of one or the other of these nerves. Both vagi nerves were then cut a short distance above the diaphragm, and also both phrenic nerves.

The stimulations invariably incited typical depressor reactions which, however, possessed a smaller amplitude than those obtained previous to the division of these nerves (fig. 4). No doubt, this change is due in some measure to the low bloodpressure occasioned by the division of the sympathetic fibers, because it was possible to again render them more conspicuous by injecting a small quantity of normal saline solution. While these results do not minimize the importance of the abdominal organs as factors in the production of this reaction, they clearly prove that the latter finds its origin in a reflex vasodilatation which involves the circulatory system in general.

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LOCALIZATION OF A PRIMARY PACEMAKER IN THE TURTLE'S HEART

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INTRODUCTION

This work was undertaken in an attempt to obtain evidence regarding the place of origin of the cardiac impulse in the turtle's heart.

HISTORICAL

The specialized tissue of the mammalian heart has been warmed and cooled by a number of workers since MacWilliam (1), and Adam (2). Until very recently such work has not been done upon the cold-blooded heart. There has been no attempt, except recently, to see whether there is a particular spot or portion in the sinus venosus of the lower animals which not only has fundamental significance in heart activity, but which would necessarily be carried forward or rather be embodied in the cardiac mechanisms of animals higher up in the scale. Certain workers have questioned the sino-auricular node as the seat of impulse formation in the mammalian heart unless it could be shown that the homologous structure of the cold-blooded forms had a similar function. The general conception has been that it did not matter what part of the sinus venosus was incorporated (developmentally in the mammalian heart) because it was thought that that in itself was sufficient, seeing that the sinus venosus (since the work of Gaskell, MacWilliam, and Engelmann), has the highest degree of automaticity of the heart. Erlanger (3) notes that in lower forms the pacemaker does not begin in the sinus node. Meek and Eyster (4) have briefly described the views on the origin of the heart-beat in cold-blooded hearts. Their own results "indicate that the point of primary negativity is in the region of the right cavo-sinal junction." They later published a preliminary communication on the origin of the cardiac impulse in the turtle's heart, "and found the junction to

precede in negativity." The beat was initiated not in the entire sinus but at a definite spot, which they have further definitely located, in 1916 (5). They were the first to do for the cold-blooded heart, what Wybauw, and Lewis, Oppenheimer and Oppenheimer, did for the mammalian heart. This spot has been identified as identical histologically with the specialized tissue in the sino-auricular junction of lower forms by Keith and Flack, Keith and Mackenzie, and Laurens (see 5).

Definite localization of beat initiation of the frog's heart was undertaken by Engelmann, in 1896 (6). He concluded that the heart-beat does not arise from any definite point in sinus or veins but that any portion in them can function as pacemaker. Engelmann (7) later (1903) reaffirmed his first position.

Engelmann is the only worker who has warmed (with platinum galvano-cautery) or influenced circumscribed areas of the sinus venosus and veins in the cold-blooded heart (frogs—normal and atropinized). Gaskell was the first to heat or cool the whole sinus (turtle) and conclude therefrom that the beat originates in it.

An advance is made when cold, warm, electrical and other stimuli under definite control are applied to closely circumscribed areas of the heart, especially in the sinus venosus.

THE PROBLEM

We attempted to find a spot in the veins, sinus venosus proper, or the sino-auricular junction of the turtle's heart, which upon being properly cooled, warmed, stimulated directly by an electric stimulus, or seared, would change the rate of the heart, or stop it altogether or temporarily.

METHODS

Experiments were performed on turtles, most of them 10 and 12 inches long, all of which had been double pithed. The plastron was removed, the pericardium incised, and the frenum cut through so that the heart could be examined dorsally and ventrally. In some cases the aortae were ligatured, and cut, to free the heart entirely. In one group, the contractions of the right auricle and ventricle were recorded by air transmission through receiving tambours to recording tambours. In this group and those following, various spots on the sinus, auricles, and veins, were cooled—in some experiments with ethyl chloride, in others with pencils of ice, in some by means of a

copper tip attached to an apparatus from which one could also supply the tip with warm water when needed and so warm it; and in others the spots were stimulated directly by an electric current, or warmed by the copper tip. The temperatures applied by the copper tip were carefully noted. In a second group of experiments the contractions of the sinus and right auricle were recorded directly on the drum by way of a vertical thin glass rod, with cork tip resting on the chamber, meeting a horizontal recording lever. In a third group, the various spots of the heart were seared by a red-hot rod. In a fourth group, the rate and action of the chambers were counted by direct inspection and observation, with no records being made. This was done on the heart in situ, and on the excised heart immediately after excision, or twelve to twenty hours after excision. In Experiment XX, and a few others, the ventricle was removed entirely after being ligatured at the auriculo-ventricular junction, and a forceps was placed on the auricles so that the sinus venosus itself received the entire attention of the observers. In some experiments atropine and nicotine solutions were placed on the heart directly; and in Experiment XXV, 1 per cent solutions of atropine, apocodeine, and nicotine were used.

We have felt justified in interpreting the change in rate produced by the various stimuli on the turtle's heart as an effect upon specialized tissue, because previous experiments on the mammalian heart rigidly controlled by histological examination have proven such assumption to be correct for the mammalian heart. Histological examinations of the tortoise heart show that the best connection between the sino-auricular ring and the right auricle is at the position of the right venous valve.

The first experiment was made by Mr. Schlomovitz, in the laboratory of Prof. A. S. Loevenhart, University of Wisconsin, on July 13, 1914. Five were completed there. Experiment VI and all the rest were concluded at Iowa, starting October 19, 1914, and from then on the authors were jointly interested in the work.

EXPERIMENTAL RESULTS

Figure 1 shows how the various spots were designated, and illustrates the elimination method of spots on the heart chambers until the definite one was determined which could be influenced markedly.

A. Temperature influences—heart in situ

Cooling. A definite spot was found which, upon being cooled by ethyl chloride spray, decreased the rate of the heart (fig. 2, Experiment II). This spot we have tried to localize as definitely as possible, but of course it may be possible that the cooling effect may have spread not only to the right of the median line, dorsal side, but into the adjacent sino-auricular junction. This same error may hold throughout the experiments, although at times we were able to cool a spot not more than 2 mm. in diameter with complete assurance that there

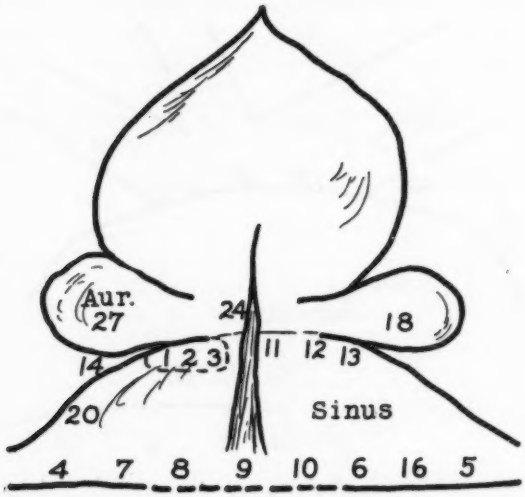

Fig. 1. Dorsal view of turtle's heart. Figure referred to in text.

was no spreading. In Experiment XVIII (at No. 18), slowing was produced by applying an ice pencil, and in Experiment XIX at (No. 37) the rate was decreased 50 per cent for a short period. Figure 3 shows the outlines of the sinus and the spots warmed, cooled, etc. In this experiment (XX), the ventricle was cut away after applying a ligature in the auriculo-ventricular junction, and a forceps placed upon the auricles, in order that the sinus movements could be accurately recorded, and certainly not interfered with by the strong ventricular pull.

Fig. 2. Effect of localized cold on turtle's heart. Cooling with ethyl chloride. Upper record, contractions of auricle. Lower record, contractions of ventricle. Time in seconds.

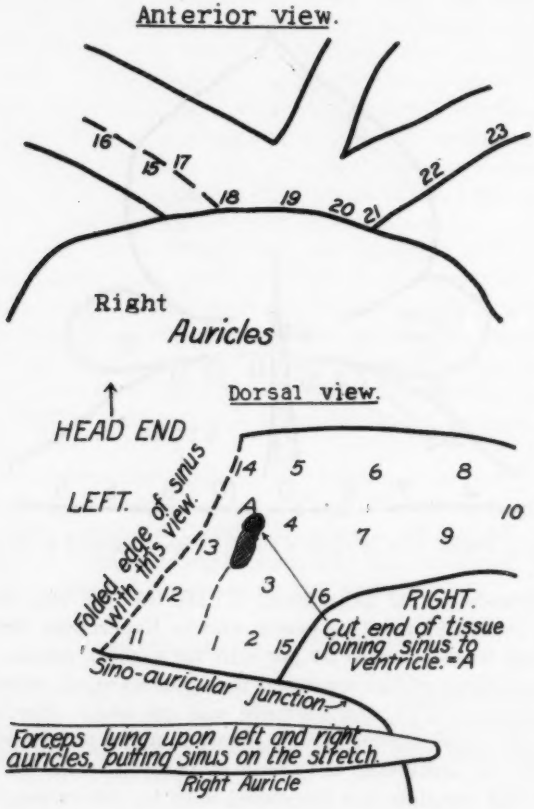

Fig. 3. Sinus region of turtle's heart showing areas warmed and cooled in Experiment XX.

Warming. Warmth 35 to 40°C. produced an immediate increase in rate of the heart only when applied at the definite spot determined for that animal. This spot was in nearly each case either in the sinus venosus on the right of the median line in the S-A junction or just adjacent to it. In Experiment XX the normal rate of the sinus was 30 per minute. When spots 1, 5, 6, 7, 10, and so on, except 2, 3, and 4, were warmed, the heart rate remained 30 per minute, but at spots 2, 3, and 4, the heart rate was 48 and 54 per minute an increase of 60 and 80 per cent. In counting rates a stop-watch was used. The effective spot in this experiment is in the body of the sinus. This is irregular. When the tissue connecting the sinus and ventricle was cut at the beginning of the experiment, the auricles fibrillated for ten minutes, and then became regular again. Probably we have ourselves to blame for not exploring the S-A junction to the right of point 11 more carefully.

Table 1 is a summary of the results shown in figures 4 to 7.

TABLE 1

	EXPERIMENT	NO.	PROCEDURE	RATE PER MINUTE NORMAL	RATE DURING PROCEDURE	CHANGE IN PER CENT
Figure 4.....	XVI	84	Cooling	18	12	33
Figure 5.....	XX	53	Warmed	27	36	33
Figure 6.....	XX	36	Warmed	27	27	0
		37	Warmed	30	48	60
		38	Warmed	30	30	0
Figure 7.....	XVIII	21	Warmed	27	54	100

Excised heart. After investigating the heart's action in situ in Experiment XVIII, it was excised, and by observation no increase in the rate of auricles, ventricles, or sinus, occurred after warming a number of spots, but when a certain spot in the sinus was warmed, there was an immediate increase from 36 to 54 per minute (50 per cent). In Experiment XIX, with the heart in situ, the increase was from 30 to 54 per minute (80 per cent). The increase in rate that occurred in every case of warming with temperature 35 to 40°C., in each experiment, was rapidly, almost immediately initiated; and the increase in rate is so apparent as to almost clinch the conclusion of the observer that at that spot certainly a different kind of cardiac activity is occurring, or else a different kind of cardiac tissue is being influenced.

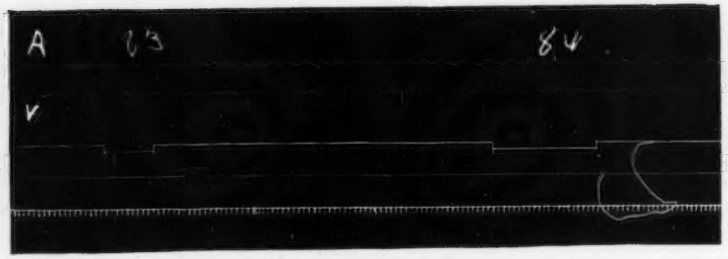

Fig. 4. First line, contractions of auricle. Second line, contractions of ventricle. Third line, signals duration of warming and cooling. Time in seconds.

Fig. 5. Rate before 27 per minute; rate during 36 per minute.

Fig. 6. Rate before No. 37, 30 per minute; rate during No. 37, 48 per minute.

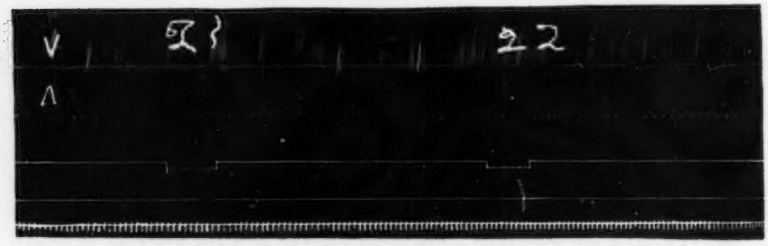

Fig. 7. Rate before—27 per minute; rate at No. 21, 54 per minute.

With an increase in temperature, 45 to 55°C., more than one spot can be found in the sinus and veins which cause an increase in rate, but there is only one spot, the sino-auricular, in which the increase is produced immediately without any latent period. In Experiment XXIV application of warmth only at the junction immediately produced fibrillation of the auricles, while at the other points including the right vein the same temperature usually produced a slight increase after a latent period.

B. Effects of atropine, apocodeine, and nicotine

In two experiments, XXIII and XXIV, the same results by warming were obtained with the heart as before, after atropine and nicotine were applied directly. In Experiment XXV, atropine, apocodeine, and nicotine were used successively in 1 per cent solutions upon the heart tissue directly. Before any drugs were applied the heart rate was doubled (100 per cent) when the thermode with temperature of 38°C. was placed on the S-A junction. There was no increase at any other point with that temperature. With temperature of 50°C. at other points on the sinus an increase in rate ensued also. After atropine was applied the increase by means of 40 to 50°C. temperature at the junction was 38 per cent, while there was no increase in rate at any other point. After the apocodeine was applied temperature effects were obtained only at the junction. In one case an increase of 43 per cent was noted. The rate now became slower gradually from 48 to 36 per minute, and then the nicotine was applied. The heart rate then dropped to 30 per minute. The auricles were beating feebly. Warming (49°C.) the junction increased the rate up to 100 per cent, while doing the same at a few other points increased the rate only 20 per cent.

Temperature effects on auricles and ventricle. No point on auricles or ventricle could be found which upon being warmed produced a change in rate.

C. Direct electrical stimulation

On the heart in situ, or excised, a series of observations were carried out in which various spots were directly stimulated by an electrical current, and we found that the least minimal electrical stimulus that will produce stoppage in a certain area will not do so at any other point. In this way, we often corroborated the results obtained by the tem-

perature method. It was also determined that the strength of current is important in making a selective localization, because there is a tendency for a current to spread, and stoppage will then be obtained at a number of points. Figure 8 taken from Experiment XX is an example of this phenomenon.

In Experiment XXIV, direct electrical stimulation produced no effect after atropine and nicotine were applied directly to the sinus.

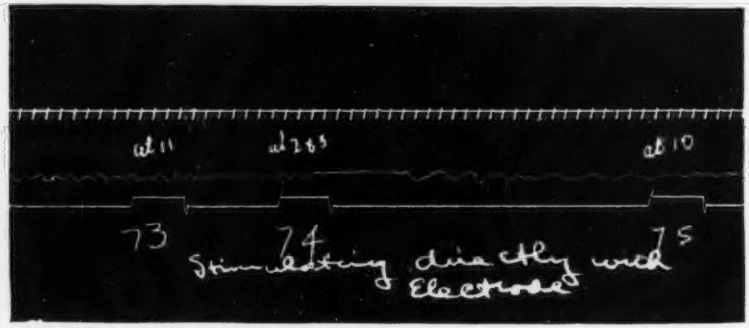

Fig. 8. For description see text

D. Cauterization

When a heated iron tip was applied to the auricle (Experiment XXI) there was at once a temporary increase in the rate due to the heating of the auricular tissue and the sinus adjacent, but when the effective spot was scalded, or seared, the heart became irregular, and its irregularity was far more evident with further scalding (Nos. 31, 46). With completer destruction of this spot the heart became very slow, thus simulating greatly the phenomenon that occurs with removal of the pacemaker from the upper parts of the specialized tissue in the mammalian heart, and the assumption of primary automaticity at some lower point in the specialized tissue.

DISCUSSION

1. *Temperature influences.* In the historical review above, it is noted as "well known that the automaticity of the heart, measured by its rate of stimulus production varies with the temperature . . . This influence of change of temperature is confined to that region

which is at the same time initiating the impulse or acting as 'pacemaker' for the whole heart" (8).

The principle laid down by Gaskell and Engelmann and supported by Hering, that the most automatic part of the heart is the pacemaker at that given moment, may explain the slowing produced in the turtle's heart by cooling. Other points in the heart have less automaticity and initiate slower rhythms, therefore, when the primal spot is cooled, it is depressed, and primary automaticity is assumed at some other, and lower point. Each part of the specialized tissue probably has its own maximum and minimum rate, so that it is also possible to cool and warm the dominant spot, just enough, without having the pacemaker change its position.

Our results show that a distinction must be made as to the degree of temperature used. The results are quite different at 35 to 40°C. than at 45 to 55°C. At the lower range only one effective point could be found, while a number of them could be found at the higher range. In both ranges the dominant spot revealed itself by being affected immediately. At other points the change set in after a latent period, which is in agreement with Engelmann's (6) observations on the frog. He heated by means of a galvano-cautery, specifying that he did not cauterize the tissues. This makes it impossible to say what temperature was used, but our observations lead us to surmise that he obtained his results by a temperature which was in our higher range or even above. He states that the pacemaking function was assumed after a latent period by a vein after intenser warming. We have found the same to be true, and as a rule it was the right vein. MacWilliam (1) had previously noted that in the hearts of cold-blooded animals there is abundant evidence of high spontaneous rhythmic power constantly held in abeyance, and that the rhythm of the portion possessing higher spontaneous rhythm will supersede the inherent rhythm of the other parts and determine the rate of contraction in the whole organ. We believe that intense warming at practically any spot in the sinus and veins, as stated by Engelmann, will result in its assuming the pacemaking function because of the fact that the warming makes the tissue more automatic. Such a shift in pacemaking function by the temperature method has been demonstrated in the mammalian heart, and it has been shown that the pacemaker remained in some part of the specialized tissue. In the mammalian heart none of the workers have found points on the vena cavae where stimuli, especially thermic, could produce typical changes in heart activity, while they

have again and again noted that the S-A junction, especially the node, was a most sensitive area, at which point heart activity could be influenced most surely, quickly, and typically.

The higher degree of temperature must have a much more striking influence in the turtle than in the mammal. The body temperature of our turtles varied from 18 to 20°C., while the highest temperature used on them varied from 45 to 55°C., giving a temperature difference of 27 to 35°C. The body temperatures of the mammals under experimental conditions were probably about 37°C. or possibly a little lower. Ganter and Zahn used a thermode at 50 to 60°C. (Adam 40 to 60°C.) which gives a temperature difference of 13 to 23°C. The highest difference used on the mammal is lower than the lowest difference for the turtle. Such a difference is important from the standpoint of the amount of heat that can be absorbed by the tissue in a given time. This may possibly explain why no typical results, if any at all, were obtained in the mammal on the veins; and probably results will be obtained if much higher temperatures or longer intervals of warming are used on the remnants of the sinus venosus, especially the termination of the superior vena cava.

Whether the difference produced by the two ranges in temperature is due to the fact that the tissue which can be influenced comes closer to the surface in the S-A junction as compared to other regions, we do not know. We doubt whether such a criticism could be maintained when the fact is considered that the tissue in the sinus and the veins is so thin, and ought to be easily influenced if there were no special mechanism for the initiation of the impulse. The problem of change in position of the pacemaker must be investigated in another way, at present best by the electronegative method.

Our results with the wider range of temperature indicate that a new seat of impulse formation in the region heated is produced by increasing the automaticity in this region. In the location of a cardiac pacemaker by warming it would seem to be important that the temperature range be small.

2. *Contractility and rhythmicity.* Engelmann emphasizes the fact that the veins had the power of contractility to a high degree, and that their power of rhythmicity was similar to that of the sinus. We believe that the ranges in temperature have revealed differences in rhythmicity The S-A junction which probably does not contract at all is most rhythmical. In their observations on dying mammalian hearts by the electronegative method Eyster and Meek (9) say, "This

suggests a possible differentiation of the properties of automaticity and contractility in certain regions of the heart. Our results merely show that impulse initiation (automaticity) may be present to almost normal extent (as judged by the rate of impulse formation) in regions in which contractility is greatly reduced or possbily absent."

3. *Cauterization.* In regard to the slow rate of the heart after searing we assume that less automatic portions became effective. Lohmann (10) was the first to conclude that abolition of the primary pacemaker in the mammalian heart made the activity take place in the A-V boundary, and his work has been confirmed by a number of workers. They have noted that as long as the sinus node was not badly injured it could again temporarily take up the function of impulse formation. This is apparently what happened in Experiment XXI, where for periods the slow rhythm was replaced by a faster one, or when warming the remains of the sino-auricular nodal tissue the latter was made more sensitive, and losing its depression, again assumed automaticity. No stoppage of the heart occurred, as so often happens in the mammalian heart, but there is such a mass of special tissue present in the cold-blooded heart, in the shape of the entire sinus venosus especially, that it seems as if there would always be some bit that would remain effective.

4. *Electrical stimulation.* Attention has been called to the fact that a difference in results ensues when the strength of direct electrical stimulation is varied The fact that a minimal strength is effective in only one region, the S-A junction, is significant. Gaskell (10) ventured the assumption that, "The probability is great that the automatic rhythm of all parts of the heart can be inhibited by the vagus nerves, when those nerve fibers are stimulated which supply directly the muscular fibers upon which the automatic rhythm in question depends." Flack (11) in "An investigation of the sino-auricular node of mammalian heart" obtained marked inhibition of both auricles and ventricles with weak stimulation of the node, but at no other point. We have data, to be reported later, which seem to indicate that the vagal endings exert great influence at the S-A junction. The S-A node when functioning as pacemaker in the mammalian heart has been shown to be intimately related with the vagi, and this seems to be true in the cold-blooded heart also.

5. *Localization of the pacemaker after drug application.* MacWilliam and Engelmann obtained their results on atropinized as well as normal animals. Our results were identical after the use of solutions of atro-

pine, apocodeine, and nicotine (to eliminate nerve influences as far as possible), in that the pacemaker was still in the S-A junction. It was noted that a higher temperature was needed to get results, indicating that the tissue had been depressed by the drugs.

Conclusion. Erlanger in particular has voiced the view that the S-A node in the mammal cannot reasonably be considered as the seat of impulse formation unless the same were true of the homologous structure in the cold-blooded animal. The S-A junction in the cold-blooded forms has been shown to be mostly nodal tissue. Meek and Eyster (5) have shown, as we have also, that the cardiac impulse originates in this definitely localized position in the turtle's heart, so that there is no more a distinction between cold- and warm-blooded hearts from this standpoint. "All vertebrate forms seem to agree in having the pacemaker of the heart located at the sino-auricular junction in masses or nodes of specialized tissue" (5). The rationalism demanded by Erlanger and others has therefore been justified.

SUMMARY

1. (a) Slight warming or cooling of the right sino-auricular junction or the parts adjacent to it, usually on the dorsal aspect, will increase or decrease the rate of the heart respectively. This change is produced only at this spot with a temperature range of 35 to 40°C.

(b) With a temperature range of 45 to 55°C. a number of points on the sinus and veins could be influenced to produce a change in rate

(c) In localization of a primary pacemaker by the temperature method the temperature range should be close to normal.

2. The application of atropine, apocodeine, and nicotine does not abolish the influence of temperature at the junction.

3. Stimulating the junction directly by a minimal electrical current will slow or stop the heart—and only at the junction. A stronger current applied to a number of points in the sinus and veins will slow or stop the heart.

4. The primary pacemaker in the turtle's heart has been shown to be in a definitely localized portion, and not the whole sinus venosus.

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THE CONDITIONS OF PHYSIOLOGICAL CONDUCTION IN IRRITABLE TISSUES

III. Electrolytic Local Action as the Basis of Propagation of the Excitation-Wave

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In my two recent papers on conduction[1] I have advanced the theory that the transmission of the excitation-state from the immediate site of activity to the adjoining resting areas is dependent on an *electrical local action* of the same essential nature as that which is responsible for the etching or corrosion of non-homogeneous metallic surfaces (*e.g.*, of iron) in contact with an electrolyte solution. Under such conditions—as is now well established—local couples are formed between those adjoining portions of the metallic surface which differ in composition or physical condition and hence in solution-tension, one region, that of higher solution-tension, forming the local anode, the other the local cathode. These local areas are the seat of electrolytic effects; at the local anode (where the positive stream enters the solution) the metal undergoes solution and chemical alteration (rusting in the case of iron), while at the cathode hydrogen (or other cation of similarly low solution-tension) is usually evolved and alkali set free. This cathodal deposition of hydrogen may incidentally have a polarizing effect and limit the current, hence the favorability of free oxygen or other depolarizing agent to the corrosive process, which is thus essentially electrolytic in nature.[2] The existence of

[1] This Journal, 1914, xxxiv, 414; 1915, xxxvii, 348.

[2] This theory is originally due to Faraday and de la Rive: "The whole subject of corrosion is therefore an electrochemical one, and the rate of corrosion is simply a function of electromotive force and resistance of circuit" (Whitney: Journ. Amer. Chem. Soc., 1903, xxv, 394). The literature is very large; some of the more important recent papers are cited below. For a discussion of the part played by depolarizing agents (in relation especially to the practical problem of protecting iron from rust) *cf.* Walker and Lewis: Journ. Ind. and Engin. Chem., 1909, i, 754.

these local chemical effects may be most readily demonstrated in the case of iron by the use of the "ferroxyl" reagent of Walker;[3] this is a solution containing phenolphthalein and ferricyanide and thickened by gelatine to retard diffusion; when this reagent is applied to the surface of ordinary iron, the anodic areas turn blue (from iron ferricyanide) and the cathodic areas red (from the alkali set free). Such local areas are more numerous in non-homogeneous than in homogeneous specimens of iron; the reason why the former rust so much more readily thus becomes clear. Rusting is in fact due chiefly to the electrolytic action of local circuits; such circuits are naturally more numerous when the surface has an irregular composition, with regions of lower and higher solution-tension. This is why perfectly pure iron is so remarkably resistant to corrosion.[4] Other conditions than simple chemical purity may however enter as factors; thus regions of mechanical strain have usually higher solution-tension than unstrained regions,[5] hence local mechanical treatment or pressure may cause rusting in pure iron;[6] the peculiar condition known as "passivity" may also play a part.[7] The dependence of rusting on local electrolytic action is further shown by the rust-preventing effects of contact with metals of higher solution-tension than iron, such as zinc;[8] in this case the zinc forms the anode, the iron the cathode, and oxidation of the latter is prevented,—hence the use of zinc strips to protect boiler-tubes, propellers and other iron parts from corrosion. Conversely, contact with nobler metals accelerates the corrosion; in this case the iron is the anode or dissolv-

[3] Walker, Cederholm, and Bent: Journ. Amer. Chem. Soc., 1907, xxix, 1251.

[4] *Cf.* Lambert and Thomson: Journ. Chem. Soc., 1910, xcvii, Trans., 2426; Dunstan and Hill: *Ibid.*, xcix, 1835; Lambert: *Ibid.*, 1912, ci, 2056; Walker: *Loc. cit.*

[5] *Cf.* Desch's Metallography (in Ramsay's series of Textb. of Phys. Chem.), 1913, 286 *seq.;* Spring: Bull. Acad. Roy. Belg., 1903, 1066; Lambert: *Loc. cit.*

[6] See the instances cited in Desch's Metallography; Lambert: *Loc. cit.* Cadmium is especially sensitive to mechanical treatment; rubbing the surface of a piece of this metal makes the latter the anode when compared with an untreated specimen.

[7] *Cf.* Mugdan: Zeitschr. f. Elektrochem., 1903, ix, 442; Dunstan and Hill: Journ. Chem. Soc., 1911, xcix, 1835; Lambert: *Loc. cit.;* Grave: Zeitschr. f. phys. Chem., 1911, lxxvii, 513; MacLeod-Brown: Chem. News, 1913, cvii, 14; Grube: Chem. News, 1913, cviii, 259, 271, 283.

[8] For experiments illustrating this effect *cf.* Dunstan, Jowett, and Goulding: Journ. Chem. Soc., 1905, lxxxvii, Trans., 1548.

ing electrode; accordingly tin-plated or nickel-plated iron rapidly rusts wherever the outer coating is broken through.[9]

It is obvious that these facts have a highly general bearing; they apply in greater or less degree to all metallic surfaces in contact with electrolytes; and since all such effects depend primarily on the electromotor properties of the surfaces—i.e., their ion-combining or ion-liberating power—they must apply also to other surfaces, non-metallic in character, which have analogous electromotor properties—in all probability to all phase-boundaries across which ionic transfer of electricity is possible.

The intimate bearing of these and similar facts upon the conditions existing at the surface-films of living cells is plain. In my second paper I compared the living irritable element with an iron wire covered by a homogeneous film of pure zinc and immersed in an electrolyte solution;[10] such a wire is in chemical and electrical equilibrium and shows no change. But any interruption of the zinc coating sets up a local iron-zinc couple, and chemical and electrical effects at once result. The case of the film-covered surface of mercury in hydrogen peroxide solution (in the rhythmical catalysis of Bredig) is essentially similar, and offers closer biological analogies; here the local electrolytic process initiated by the local interruption of the film causes the dissolution of the film-substance, and hence the effect is propagated over the whole surface of the mercury.[11] Similarly in the resting irritable element, *e.g.*, nerve-fibre, the unaltered plasma-membrane represents a homogeneous surface-film of uniform electromotor properties; the local stimulating agent causes locally a change in the electrical condition of the surface; the potential-difference between protoplasm and medium is decreased at that point (in consequence apparently of the increased permeability of the film), and a local couple arises, with the current flowing in the external medium from the unstimulated to the stimulated region. According to the hypothesis advanced in the paper cited, this local circuit, having once originated, produces effects similar to those of any other electrical current upon the adjoining portions of the irritable element; at the still resting regions the direction of the current entering the cell-surface from the external medium is such as to cause local

[9] The effect of contact of carefully purified iron with a noble metal (platinum) is well shown in experiments by W. A. Tilden: Journ. Chem. Soc., 1908, xciii, Trans., 1356.

[10] Loc. cit., 357.

[11] Antropoff: Zeitschr. f. phys. Chem., 1907, lxii, 513.

depolarization,[12] an effect resulting, if sufficiently intense, in stimulation. This secondarily stimulated region produces similar effects at the region beyond; hence the excitation-effect *spreads*, at a rate proportional to the rate of development of the local current and the maximal distance (from the original area of excitation) through which its stimulating effect is felt.

This theory, which I propose to call "the local action theory" of conduction, evidently assumes that the electromotor properties of the protoplasmic surface-film are determined by conditions which are fundamentally similar to those governing the electromotor phenomena at metallic surfaces. That this is actually the case is clearly indicated by the observations of Macdonald,[13] Loeb and Beutner,[14] and Beutner[15] on living cells, organic membranes, and solutions of lipoids in organic solvents. The surfaces of such systems behave as if reversible to cations as a class, and the law of Nernst applies; any such surface must therefore be regarded as possessing, like a metal, an electrolytic solution-tension,—one, however, which presumably varies with the physical and chemical condition of the surface-film.[16] Variations in the solution-tension of such a protoplasmic surface-film would produce essentially the same electrical effects as variations in the solution-tension of a metallic surface. Local alteration of the cell-surface would thus give rise to local electrical circuits; these, like other currents, would exert stimulating and inhibiting influences on adjacent portions of the cell, or possibly on neighboring cells. In the present paper I wish to pursue this comparison in further detail, and to call attention to certain other analogies between the phenomena of local action at metallic surfaces and at the surfaces of living cells.

In the irritable living element the local effect differs from that in the metal in being *evanescent*, and in being propagated quickly from the original site of disturbance; *i.e.*, the change at the excitation-area is there immediately reversed, and is transmitted with greater or less rapidity to adjoining regions; in other words, it *spreads*, at a rate de-

[12] *Cf.* p. 362 of my paper cited.

[13] Macdonald: Proc. Roy. Soc., 1900, lxvii, 325.

[14] Loeb and Beutner: Biochem. Zeitschr., 1912, xli, 1; xliv, 303; 1913, li, 288.

[15] Beutner: Zeitschr. f. Elektrochem., 1913, xix, 319, 467; Trans. Amer. Electrochem. Soc., 1913, xxiii, 401; this Journal, 1913, xxxi, 343.

[16] This, however, is also true for metals; witness the difference between active and passive iron, the two varieties of tin or chromium, strained and unstrained metals.

termined by the temperature, the composition of the medium, and the specific peculiarities of the tissue. The problem of the physico-chemical basis of this "conduction" requires for its solution a determination of the general conditions under which surface-changes of this kind spread from active to inactive regions, whether in a living cell or an inorganic system.

It is first to be noted that spreading effects, showing in many ways close analogies with those of living tissues, are frequently met with in simple inorganic systems of the above class. The case of the film-covered mercury-surface in hydrogen peroxide solution is an instance which I have already cited in former papers.[17] Other interesting analogies are seen in the spreading of the "active" condition over the surface of "passive" iron or other similar metal in consequence of a local alteration which may, as in the case of living tissues, be of a mechanical, chemical, or electrical kind. The passivity is thus locally destroyed, and the effect spreads over the whole surface. This phenomenon is thus described by Dunstan and Hill.[18] Iron in the passive state differs from normal iron in not being attacked by nitric acid (s.g.1.2), but remains bright and unaffected in the solution; it is however at once attacked if touched with a piece of active iron, or scratched with a glass rod; the action then visibly spreads from the point of contact over the whole surface. Similarly a solution of $CuSO_4$ (0.5 per cent) deposits a film of copper on active iron, but not on passive iron, which remains bright until touched with active iron or scratched; the film of copper can then be seen to spread from the region of contact. A mechanical jar is often sufficient to activate a piece of passive iron, or local bending or pressure;[19] electrical currents, especially if of high density, may have a similar effect.[20] In all of these effects the destruction

[17] This Journal, 1909, xxiv, 18; 1914, xxxiv, 426; Popular Sci. Monthly, 1913, 151.

[18] Dunstan and Hill: Journ. Chem. Soc., 1911, xcix, Trans., 1835, 1853. See also Lambert's description of the behavior of pure iron in solutions of copper salts; copper is only slowly deposited on pure iron from dilute $CuCl_2$ solutions; dull spots appear on the bright surface of the iron and the effect spreads quickly (p. 2070). Similarly spots of rust on a bright iron surface tend to spread, the deposit having, as it were, an autocatalytic influence. The rusted region acts as cathode relatively to the unrusted regions which, as anodes, tend to dissolve, etc.

[19] *Cf.* Dunstan and Hill: *Loc. cit.;* also Lambert: *Loc. cit.*

[20] *Cf.* Hayden: Journ. Franklin Inst., 1911, 295. Active chromium is rendered passive by having a strong current pass through it as anode (Le Blanc: Zeitschr. f. Elektrochem., 1900, vi, 472). The same is true of nickel, and apparently also of iron (Le Blanc: Boltzmann Festschr., 183).

of passivity and the transmission of the active condition are almost certainly due to an alteration of solution-tension and the formation of local electrolytic couples. Passive iron has a lower electrolytic solution-pressure than active iron.[21] The activated region thus becomes the anode in the local circuit; the region still passive is the cathode; the current thus passing alters the condition at the temporary cathodal areas which are thus rendered active—possibly by the nascent hydrogen there freed—and so the effect spreads until the whole surface is activated. The transmission of activation in metals is thus due to electrical conditions,—just as in the living system, according to the present theory. Both effects may in fact be regarded as autocatalyses of a special kind.

This brief description does not pretend to discuss the phenomena of activation and passivation in metals in any detail, but merely to call attention to the more important biological analogies. The spreading of the effects of stimulation or other local alteration is one of the most characteristic peculiarities of living systems; it is in fact essential to any form of stimulation. In any such process a propagated disturbance[22] is set up by some form of local alteration; in this way the whole cell or element is activated. The local action theory attributes this effect to the formation of a local circuit at the original site of alteration; by the action of this circuit the altered condition is automatically transmitted to adjoining regions. Some of the most striking recent examples of such transmission are afforded by the observations of Kite and Chambers on the effects following puncture or tearing of the surface-layers of various cells by microscopic glass needles. A leucocyte or red blood corpuscle thus locally injured undergoes rapid and complete dissolution;[23] many other cells show similar changes;[24] the effect of the local injury spreads from the altered region over the

[21] LeBlanc: Boltzmann Festschr., 1904, 183; Mugdan: Zeitschr. f. Elektrochem., 1903, ix, 442; Grave: Zeitschr. f. phys. Chem., 1911, lxxvii, 513; MacLeod-Brown: Chem. News, 1913, cvii, 14; Grube: Chem. News, 1913, cviii, 259, 271, 283.

[22] This term of Keith Lucas (Journ. Physiol., 1912, xliv, 68) is applicable not only to the transmission of the excitation-state, but also to the spreading of the effects of local injury in cells (about to be described), as well as to the propagation of the active state over the surface of passive metals.

[23] Chambers: Lancet-Clinic, March 27, 1915; *cf.* also Oliver: Sci., 1914, N. S., xl, 645.

[24] Kite describes such effects for Amœba and Paramœcium (this Journal, 1913, xxxii, 146); Chambers for the germ cells of insects (Sci., 1914, N. S., xl, 824; 1915, xli, 290).

entire cell-surface; sometimes the entire cell goes into solution with almost explosive rapidity.[25] It is clear that the local injury initiates a disturbance which is rapidly propagated and results in the destruction of the osmotic properties of the whole surface-film; Chambers observes that when a red corpuscle is punctured the haemoglobin diffuses out; "it is significant that the outflow of the haemoglobin does not take place only at the punctured spot, but over the entire surface of the corpuscle."[26] In such phenomena we have instances of transmission of an altered condition of the surface-film—involving evidently a marked increase of permeability—which are probably identical in their general characters with the transmission of the normal excitation-state in irritable elements. Both phenomena represent special instances of protoplasmic conduction, although in the one case reversible, in the other irreversible, effects are produced. The analogies to the transmission of the altered state of the metallic surface in the above cited experiments of Dunstan and Hill are evident. In both cases we have to do with "spreading phenomena" initiated by a local alteration; in the case of the metal the effect is clearly due to electrical circuits resulting from local changes in the electromotor properties of the surface; the same is the case, according to the present theory, in the transmission of the effects of local alteration of the cell-surface.

In the case of metals the conditions determining the rate at which the activation-effect is transmitted have not yet been fully ascertained; but it is evident that since the local electrolytic process is the primary feature of the whole phenomenon, the rate of this process must determine the rate of transmission. The velocity of the electrolytic process in any such local couple (or in a galvanic cell of any kind) depends (1) upon the potential-difference between the anodic and cathodic areas (*i.e.*, the difference between the electrolytic solution-pressures at the two regions), and (2) upon the electrical resistance of the circuit. Ericson-Aurén and Palmaer give the formula, $V = f\frac{x}{C}e$, where V is the velocity of solution of the metal (*e.g.*, iron) at the local anode, f a proportionality factor, x the conductivity of the adjoining solution, e the potential-difference, and C the resistance-capacity of the arrange-

[25] *Cf.* Kite: Loc. cit., 156; Chambers: Loc. cit., 1915.

[26] Chambers: Proc. Amer. Assoc. Anatomists (abstract in Anat. Record, 1916, x, 190).

ment (*i.e.*, the resistance attained while the reaction is proceeding[27]). If, as we must assume, the local electrolytic process has to reach a certain stage before the effect is transmitted to adjoining areas, the rate of this transmission will also depend upon the same conditions,—*i.e.*, will be proportional to the rate of the local action. This explains why the transmission of the active state, as well as the rate of corrosion in general, is more rapid in electrolyte solutions than in pure water.[28]

In a conducting tissue like nerve analogous conditions must be presumed to enter. The intensity of the local effect will depend upon the potential-difference (equivalent to difference of electrolytic solution-tension) between the active and the inactive areas, and upon the electrical conductivity of the circuit. As I pointed out in my last paper, the intensity of the current traversing the cell-surface at a point R, distant by the interval SR from the original point of stimulation S (which corresponds to the local cathode in the wire model), is dependent on the resistance of this part of the circuit.[29] Hence if a certain critical intensity of current is required for local stimulation at any point, the rate at which the excitation-effect is transmitted from the excited region S to an adjacent unexcited region R should depend upon the electrical resistance of the circuit formed by the intervening stretch of irritable element and the medium bathing it. A relation of direct proportionality should thus exist between the electrical conductivity of the medium and the rate of propagation of the excitation-wave. Whether or not such a relation is general in conducting tissues has not yet been systematically investigated. The recent observations of Mayer,[30] however, on the rate of conduction in the nerve-net of the medusa Cassiopea in dilute sea-water, seem to exemplify clearly just this kind of relationship—although not interpreted by Mayer himself in this sense. The parallelism between the rate of propagation of the contraction-wave and the electrical conductivity of the medium (with dilutions ranging from 5 to 50 volumes per cent distilled water) is remarkably close. In any such investigation a

[27] Ericson-Aurén and Palmaer: Zeitschr. f. phys. Chem., 1901, xxxix, 1. *Cf.* also the other papers of these authors: *Ibid.*, 1903, xlv, 182, and 1906, lvi, 689.

[28] *Cf.* Dunstan and Hill: Loc. cit., 1856. For the relation between the conductivity of the medium and the rate of corrosion *cf.* also Ericson-Aurén and Palmaer, Whitney, Lambert and Walker in the papers above cited.

[29] Loc. cit., 360.

[30] A. G. Mayer: Proc. Nat. Acad. Sci., 1915, i, 270 and 1916, ii, 37; this Journal, 1916, xxxix, 375.

tissue whose properties are not essentially altered by considerable changes in the osmotic pressure of the medium is needed, and the physiological balance of the salts in the medium should remain unchanged. These conditions are apparently met in Mayer's experiments; and it seems highly probable that the diminished rate of physiological conduction observed by him in dilute sea-water is a direct consequence of the diminished electrical conductivity of the medium and of the protoplasm.[31] This is the result which the local action theory of conduction would lead us to expect. Further investigation of this relationship in a variety of conducting tissues is however needed, and I hope before long to be able to present further data on this subject.

In the transmission of the state of excitation from the active to the adjoining inactive regions in a conducting element like a nerve-fibre, the conditions appear to be essentially the same as in the spreading of the altered condition of the surface-film in the above cited experiments of Kite and Chambers. The two processes differ however in that the rate of transmission in the irritable element is usually much higher, and the effect at the active region is *transitory*, *i.e.*, the changes associated with excitation (negativity and increased permeability) are rapidly and automatically reversed when the wave of excitation reaches any region of the element. Thus in the typical irritable element like nerve the local excitation-process is automatically self-limiting. The high velocity of transmission is apparently a function of high irritability—*i.e.*, promptness and rapidity of local change, and great sensitivity to variations of surface-polarization—and its conditions have been discussed in the first paper of this series. With

[31] Mayer believes that the rate of nerve conduction is proportional to the quantity of ions *adsorbed*, rather than to the concentration of ions in solution. In the formula which he finds best to agree with his observations ($y = 2.512\, x^{0.8}$, y being the rate of conduction, and x the concentration of Na, Ca and K ions in solution in the medium), the assumed adsorption exponent, 0.8, is so near unity that the assumption of a direct proportionality between the rate of conduction and the concentration of ions in solution—*i.e.*, the electrical conductivity—gives an almost equally good agreement with observation. Possibly the dilute sea-water may somewhat impair the tissue and account for the slight deviation from direct proportionality with moderate dilutions; at dilutions above 50 per cent the rate of conduction falls off much more rapidly than the electrical conductivity.

It may be pointed out that the need for salts in the protoplasm and media of irritable tissues is in all probability largely an expression of this need for electrical conductivity (*cf.* the first paper of this series, this Journal, 1914, xxxiv, 429).

regard to the self-limiting characteristic, there is every indication that this is at bottom nothing but an expression of the well-known polar action of the electrical current upon such tissues,—*i.e.*, inhibition where the current enters the element (at the anode of the electrodes from an external source of current), and stimulation where it leaves. As shown in the diagram on page 362 of my last paper, the current of the local active-inactive circuit has such a direction that at the original site of excitation the positive stream *enters* the cell-surface from the medium, while at adjoining regions (not yet excited) it *leaves*. Since there is no reason to believe that a self-generated current will have different effects upon a tissue from one reaching the latter from outside, we must conclude that the local current, having once originated, *itself* inhibits the excitation at the active area, in addition to initiating excitation at the adjoining inactive areas. This process is automatically repeated at the new region of excitation, and so the effect is transmitted as a wave over the whole element. The present hypothesis therefore simply assumes that the action-currents themselves influence the activity of the tissues by which they are generated—and also probably that of adjacent cells and tissues[32]—and that this influence is of the same nature as that exerted by electrical currents in general, whatever their source. If this is granted, the immediate inhibition of activity at the original site of activity, and its initiation at the adjoining regions, will be seen to be automatic and necessary consequences of local activity in any irritable element having the properties of a nerve-fibre. Any local electrical circuit in a system with such properties will of necessity locally limit itself and be transmitted to adjacent regions. Hence a wave of electromotor variation travels along the surface of the irritable element.

It should be noted that this self-limiting effect is not an unexampled and distinctive peculiarity of living tissues, but is shown to a greater or less degree by all circuits having polarizable electrodes. A counter electromotive force is set up as the current starts, which diminishes the latter, to a degree and at a rate which depend on the special nature of the conditions. Thus in the case of a circuit of copper and platinum

[32] For example, it is possible that such a phenomenon as the reciprocal inhibition of antagonistic neurones in the central nervous system may be due to these conditions, as I pointed out in a former paper (this Journal, 1913, xxxi, 285). It may be that the bioelectric processes have a general coördinating influence of still wider application (*cf.* the first paper of this series, *loc. cit.*, 427 *seq.*).

in a solution of $CuSO_4$ the electromotive force gradually falls to zero owing to the deposition of copper on the platinum.[33] In the active nerve-fibre or similar irritable system we must assume that in an analogous manner some process occurs where the current enters the cell-surface—which arrests activity and restores the resting properties of the surface-film, *i.e.*, causes re-polarization; while at the region where the current leaves, the reverse process occurs, and the film undergoes an alteration involving increased permeability and change of electromotor properties; this change, however, is immediately reversed in the manner already indicated. The precise metabolic basis of these changes cannot be indicated at present; the above facts, however, imply that processes of an essentially electrolytic character play a fundamental part.

[33] It should be noted that living tissues in general exhibit a high degree of electrical polarizability; polarization-currents of a strength sufficient for stimulation arise immediately when a current is passed through such a tissue.

CRYOSCOPIC MEASUREMENT OF THE OSMOTIC DIFFERENCE BETWEEN RESTING AND FATIGUED MUSCLE

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It has been shown by Ranke (1) that when striated muscle is fatigued and put into an indifferent salt solution, it absorbs water in greater measure than a resting muscle kept in the same solution. Ranke sought to account for this increased inbibition on the part of fatigued muscle by supposing dissociation products to be formed in the tissue as the result of its activity, and this to bring about an increased attraction for water. In modern terms, fatigue increases the osmotic pressure of muscle by breaking down some of the larger molecules into more numerous smaller ones.

Other workers have confirmed Ranke's results. The method consists in immersing the muscle in a solution of known concentration from which it is removed from time to time and weighed. In this way Cooke (2) showed that the increase in osmotic pressure may be as high as 37.5 per cent as the result of fatigue. Later, Fletcher (3) found the curves of swelling of resting and of fatigued muscle to differ characteristically, the latter always reaching an earlier maximum weight. In salt solutions isosmotic with the animal's blood the fatigued muscle gained in weight but the normal control remained unchanged for hours.

In case the muscle be exercised in the body with the circulation intact, it increases in weight (4), volume (5) and decreases in specific gravity (6) indicating water absorption from the lymph. It is known that the blood flowing from an exercising muscle suffers a loss in water and an increase in solid content (7). It has been suggested by certain workers that the increased imbibition of water by an exercised muscle may be due in part to changes in the permeability of the muscle cells (8). The connection between the acidity accompanying muscle contraction and water absorption has been frequently emphasized, and

by some, acidity is considered a possible cause of muscle contraction (9).

The views referred to are sufficient to indicate the unsettled condition of the question of water absorption by muscle, and the importance of obtaining new evidence on the point at issue, (10), viz: Is the increase of osmotic pressure in a muscle, resulting from fatigue, alone sufficient to account for the increased imbibition on the part of such a muscle? It is apparent that measurements of weight increment and of specific gravity show the fact of water absorption solely, without indicating unequivocally the underlying cause. In order to determine exactly what part osmotic pressure plays in causing the phenomenon in question, it is necessary to measure the concentration of dissolved substances in the muscle, both resting and fatigued, and to compare the values obtained. Cryoscopic determinations afford a reliable method for doing this, because, in dilute aqueous solutions such measurements depend entirely upon the gram-molecular concentration and, in the case of electrolytes, the degree of ionization.

Fifteen years ago Sabbatani (11) showed that the freezing point of uninjured tissue could be determined with a fair degree of accuracy. This was done by packing the tissue around the bulb of a Beckmann's thermometer, allowing the preparation to undercool until spontaneous crystallization took place, and reading, as the freezing point, the highest mark reached by the mercury column. I have found it possible by the use of this method, to duplicate my observations with an experimental error of $\pm 0.01°$, provided the degree of undercooling be controlled by inoculating the preparation with an ice crystal.

In the following experiments large bull frogs, *Rana catesbiana*, were used. The animal was killed and the muscles of one leg removed with as little injury as possible. The superfluous liquid was absorbed from the tissue with filter paper, and the muscles packed around the bulb of the thermometer in a Beckmann's apparatus. Two or three successive readings were taken. The highest reading of each experiment, i.e., the least depression, is given in the table. Meantime the other leg of the same animal was prepared by removing the skin and absorbing the superfluous lymph with filter paper. The preparation was next placed in a moist chamber and stimulated directly with a maximal tetanizing current until completely fatigued, usually about two hours. It was then removed and the freezing point of the muscles determined as described for the other leg. Since the loss in weight of the preparation during stimulation was less than 0.5 per cent, any

appreciable lowering of the freezing point below that of the control must be attributed alone to an increase in the concentration of dissolved substances or their dissociation products, in the fatigued tissue. The following measurements show that in every case the freezing point of frog's muscle is significantly depressed by fatigue (table 1). Each numeral indicates a pair of measurements made from a single individual. Δr = depression of the freezing point for resting muscle; Δf = that for fatigued muscle; $\Delta f - \Delta r$ = increase in depression of the freezing point due to fatigue; M = gram-molecular concentration of a non-electrolyte sufficient to produce the increase $\Delta f - \Delta r$, hence $M = \frac{\Delta f - \Delta r}{1.85}$, in which 1.85°C. = depression of the freezing point of a molar solution; per cent = per cent of increase in the depression of the freezing point resulting from fatigue.

These results show that the increase in the osmotic pressure of muscle due to fatigue amounts to an average of 36 per cent which is equivalent to an increase in concentration of 0.08 mol and is of sufficient magnitude to account for the imbibition of water previously observed.

TABLE 1

	Δr −°C.	Δf −°C.	$\Delta f - \Delta r$ −°C.	M	PER CENT
1	0.43	0.54	0.11	0.06	26
2	0.43	0.56	0.13	0.07	30
3	0.41	0.54	0.13	0.07	32
4	0.385	0.545	0.16	0.085	42
5	0.41	0.565	0.155	0.085	38
6	0.40	0.58	0.18	0.095	45
7	0.405	0.58	0.175	0.095	43
8	0.39	0.57	0.18	0.095	46
9	0.41	0.55	0.14	0.075	34
10	0.41	0.58	0.17	0.09	41
11	0.43	0.58	0.15	0.08	35
12	0.42	0.56	0.14	0.075	33
13	0.45	0.56	0.11	0.06	24
14	0.43	0.57	0.14	0.075	33
15	0.45	0.62	0.17	0.09	38
16	0.46	0.63	0.17	0.09	37
Mean.....	0.42	0.57	0.15	0.08	36

The increase in water absorption from a surrounding solution by a fatigued muscle is therefore brought about by an increase in the concentration either of molecules or of ions or of both, within the tissue.

The experiments were repeated with the circulation intact. This was best accomplished by destroying the brain anterior to the medulla, and severing the sciatic plexus from the cord on both sides. This section was performed through an opening into the body cavity made by removing the urostyle. A frog prepared in this way retained its respiration and circulation. The entire animal was put into a moist chamber and one leg was fatigued by direct stimulation. The freezing points of both resting and fatigued muscles were determined and found to be identical as shown in table 2.

TABLE 2

	Δr −°C.	Δf −°C.	$\Delta f - \Delta r$ −°C.
1	0.47	0.46	−0.01
2	0.46	0.46	0.00

This result agrees with the observations of Lazarus-Barlow, Barcroft and others who have shown that a muscle, which is exercised *in situ* with the circulation intact, compensates in part for its hypertonicity by absorbing water from the lymph and ultimately from the blood. If we further suppose the circulation to remove some of the fatigue products, the identity of freezing points of resting and of fatigued muscles is to be expected.

CONCLUSIONS

1. The mean depression of the freezing point for the resting muscle of the bull frog was found to be 0.42°C.

2. The mean depression of the freezing point for the completely fatigued muscle of the bull frog was found to be 0.57°C.

3. The mean difference in freezing points for resting and fatigued muscle is 0.15°C. This is equivalent to the depression of the freezing point of an 0.08 M solution of a non-electrolyte.

4. On account of the magnitude of the increase in the depression of the freezing point as a result of fatigue, it is concluded that the increased imbibition of fatigued muscle is caused entirely by the increase in concentration of dissolved substances, ions and molecules, within the muscle.

5. The freezing point of muscle fatigued in the body with circulation intact is identical to that of the resting muscle of the same individual.

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